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(54) Title: DETECTION OF ANTI-GLYCOLIPID ANTIBODIES BY LATEX AGGLUTINATION ASSAY

(57) Abstract: The present invention comprises a method for detecting antiglycolipid autoantibodies in a subject who has or who may develop an autoimmune neuropathy. The present invention comprises a method for detecting antiganglioside autoantibodies in a subject. The present invention also provides methods for detecting multiple antiganglioside autoantibodies in a subject, simultaneously or consecutively. The present invention also provides methods for quantitating ganglioside autoantibodies in a subject. The present invention also provides a method of diagnosing autoimmune neuropathy in subjects with peripheral neuropathies. The present invention also provides a method of diagnosing autoimmune neuropathy in celiac disease in a subject.

DETECTION OF ANTI-GLYCOLIPID ANTIBODIES BY LATEX AGGLUTINATION ASSAY

This application is a continuation in part of U.S. Serial No. 09/649,229 filed August 28, 2000, the contents of which are hereby incorporated by reference into the subject application.

Throughout this application, various references are referred to within parentheses. Disclosures of these publications in their entireties are hereby incorporated by reference into this application to more fully describe the state of the art to which this invention pertains. Full bibliographic citation for these references may be found at the end of this application, preceding the claims.

BACKGROUND OF THE INVENTION

Elevated levels of serum autoantibodies directed against gangliosides are closely associated with acute and chronic autoimmune neuropathies. For example, highly elevated titers of serum IgM anti-GM1 ganglioside antibodies are closely associated with multifocal motor neuropathy (reported to occur in 20% to 85% of patients with multifocal motor neuropathy or reversible lower motor neuron disease), but low titers are commonly present in normal individuals or other diseases. Antibodies to gangliosides are implicated in the pathogenesis of several autoimmune neuropathic syndromes, including the Guillain-Barré syndrome (1, 2), and a

number of chronic peripheral neuropathies (3). These antibodies react with oligosaccharide determinants of major or minor gangliosides, which are highly concentrated in the peripheral nerves.

In several cases, the antibodies recognize oligosaccharide determinants that are shared by different gangliosides. For example, anti-GM1 ganglioside antibodies in motor neuropathy often react with the Gal(β1-3)GalNAc epitope which is shared by GD1b (4); antibodies to GD1b in sensory ataxic neuropathy recognize disialosyl epitopes shared by GD2, GD3, GT1b, and GQ1b (5, 6); antibodies to GD1a in motor dominant neuropathy recognize the NeuAc(α2-3)Gal(β1-3) moiety shared with GT1b and GM3 (7); and anti-GQ1b ganglioside antibodies in the Miller Fisher variant of the Guillain-Barré syndrome react with the disialosyl moiety which also characterizes GD3 and GD1b gangliosides among others (8).

Reflecting this, assays for the detection of anti-GM1 antibodies are therefore increasingly used in clinical practice to aid in the evaluation and diagnosis of patients suspected of having these diseases. At present, anti-glycolipid antibodies are routinely detected by ELISA, which measures serum antibody binding to purified individual glycolipids coated onto microwells (9). This assay system is relatively cumbersome, requires several days to perform, and takes place under non-physiologic conditions of temperature and serum dilution. In addition, routine testing is limited to single major gangliosides (and not multiple antibodies), and therefore may miss sera with antibodies that react with minor

gangliosides, or with as yet uncharacterized gangliosides. Alternative liposome agglutination assays have proved difficult to manipulate in terms of consistency and reproducible assays, as well as having spontaneous agglutination problems which can give false-positives, and stability problems over time.

The present invention discloses an agglutination assay for antiganglioside autoantibody detection and also discloses that anti-ganglioside antibodies can be detected in samples from subjects presenting neuropathies in celiac disease which may serve as a basis for diagnosis. The new assay described herein can serve as a rapid and effective method for detecting, quantifying or screening for anti-ganglioside antibodies in patients with acute or chronic immune-mediated neuropathies or other disease producing antiganglioside autoantibodies. It would be particularly useful for detecting antibodies that react with minor, or as yet uncharacterized gangliosides, or with epitopes shared by several different gangliosides. Further, this invention discloses a method for detecting multiple antiglycolipid antibodies simultaneously, or rapidly detecting single antibodies that bind to multiple gangliosides. A color coding method disclosed here allows titering of different antibodies simultaneously. The invention is considerably faster and more flexible than the ELISA method currently used.

SUMMARY OF THE INVENTION

This invention provides a method of detecting the presence of an antibody directed against a ganglioside in a subject comprising:

(a) contacting a liquid sample from the subject with the ganglioside, such ganglioside being affixed to at least two separate solid particles, under conditions permitting the antibody if present in the sample to form a complex with the ganglioside, which complex comprises such solid particles; and

(b) detecting the presence of any complex formed in step (a), wherein the presence of such complexes indicates the presence of the antibody in the subject.

This invention also provides a method of detecting in a subject the presence of at least two different antibodies, each of which antibodies is directed against a different type of ganglioside comprising:

(a) contacting a liquid sample from the subject with one such type of ganglioside, such ganglioside being affixed to at least two separate solid particles, under conditions permitting the antibody directed against said type of ganglioside if present in the sample to form a complex with the ganglioside, which complex comprises such solid particles;

(b) contacting such liquid sample with a different

type of ganglioside, such different type of ganglioside being affixed to at least two separate solid particles, under conditions permitting the antibody directed against such different type of ganglioside if present in the sample to form a complex with such different type of ganglioside, which complex comprises such solid particles; and

(c) detecting the presence of any complex formed in step (b) and any complex formed in step (c), wherein the presence of complexes formed in both step (b) and step (c) indicates the presence in the subject of such different antibodies.

This invention further provides the instant method, wherein steps (a) and (b) are performed simultaneously.

This invention further provides the instant method, wherein the solid particles having affixed thereto said one such type of ganglioside are the same color and the solid particles having affixed thereto said different type of ganglioside are of a different color.

This invention further provides the instant methods, wherein the antibody is directed against more than one ganglioside.

This invention further provides the instant methods, wherein the antibody is directed against one ganglioside.

This invention also provides a method of quantitating the amount of an antibody directed against a ganglioside

present in a subject comprising:

(a) contacting a plurality of identical liquid samples from the subject with the ganglioside, each such sample comprising the ganglioside affixed to at least two separate solid particles, such particles having affixed thereto a predetermined amount of such ganglioside, wherein the predetermined amount used to contact each said sample is different, under conditions permitting the antibody if present in the sample to form a complex with the ganglioside, which complex comprises such solid particles; and

(b) detecting the presence in each such sample of any complex formed in step (a), and correlating such detection of complexes in each such sample with a predefined reference standard indicative of the amount of the antibody present in the subject so as to quantitate the amount of the antibody present in the subject.

This invention also provides a method of quantitating the amount of an antibody directed against a ganglioside present in a subject comprising:

(a) contacting a plurality of liquid samples from the subject with the ganglioside, each such sample being differently diluted and such ganglioside being affixed to at least two separate solid particles, such particles having affixed thereto a predetermined amount of such ganglioside, wherein the predetermined amount used to contact each said sample is the same, under conditions permitting the antibody if

present in the sample to form a complex with the ganglioside, which complex comprises such solid particles; and

(b) detecting the presence in each such sample of any complex formed in step (a), and correlating such detection of complexes in each such sample with a predefined reference standard indicative of the amount of the antibody present in the subject so as to quantitate the amount of the antibody present in the subject.

This invention further provides the instant methods, wherein the liquid sample is human sera.

This invention further provides the instant methods, wherein the liquid sample is chosen from the group consisting of plasma, saliva, tears, mucosal discharge, urine, peritoneal fluid, cerebrospinal fluid, lymphatic fluid, bone marrow, tissue, lymph nodes or culture media.

This invention further provides the instant methods, wherein the solid particles comprise polystyrene latex.

This invention further provides the instant methods, wherein the solid particles comprise carbonsol.

This invention further provides the instant methods, wherein the ganglioside is covalently affixed to the solid particles.

This invention further provides the instant methods,

wherein the ganglioside is chosen from the group consisting of GM1, GM2, GM3, GD1, GD2, GD3, GD1a, GD1b, GT1b or GQ1b.

This invention further provides the instant methods, wherein the ganglioside comprises total brain ganglioside extract. This invention further provides the instant method, wherein the source of the extract is a bovid.

This invention further provides the instant methods, wherein the ganglioside comprises tissue ganglioside extract.

This invention further provides the instant methods, wherein the antiganglioside antibody is an autoantibody.

This invention further provides the instant methods, wherein the antiganglioside antibody is chosen from the group consisting of anti-GM1, anti-GM2, anti-GM3, anti-GD1, anti-GD2, anti-GD3, anti-GD1a, anti-GD1b, anti-GT1b or anti-GQ1b.

This invention further provides a method of diagnosing whether a subject has autoimmune neuropathy, comprising quantitating the amount of an antibody directed against a ganglioside in the subject using either of the instant methods, wherein the presence of a predefined amount of the antibody indicates that the subject is suffering from autoimmune neuropathy.

This invention further provides the instant method, wherein the neuropathy is Guillain-Barré syndrome.

This invention further provides the instant method, wherein the neuropathy is a Guillain-Barré syndrome variant.

This invention further provides the instant method, wherein the neuropathy is a peripheral neuropathic disease.

This invention further provides the instant method, wherein the neuropathy is a multifocal motor neuropathy.

This invention further provides a method of diagnosing whether a subject that has Celiac disease suffers from autoimmune neuropathy, comprising quantitating the amount of an antibody directed against a ganglioside in the subject using either of the instant methods, wherein the presence of a predefined amount of the antibody indicates that the subject is suffering from autoimmune neuropathy.

This invention further provides the instant method, wherein the antibody is directed against GM1.

This invention further provides the instant method, wherein the antibody is directed against GD1a.

This invention further provides a method of determining if a subject is predisposed to become afflicted with an autoimmune neuropathy, comprising quantitating the amount of an antibody directed against a ganglioside in the subject using either of the instant methods, wherein the presence of a predefined amount of the antibody indicates that the subject is predisposed to become afflicted with

an autoimmune neuropathy.

This invention further provides the instant method, wherein the neuropathy is Guillain-Barré syndrome.

This invention further provides the instant method, wherein the neuropathy is a Guillain-Barré syndrome variant.

This invention further provides the instant method, wherein the neuropathy is a peripheral neuropathic disease.

This invention further provides the instant method, wherein the neuropathy is a multifocal motor neuropathy.

This invention further provides a method of determining if a subject with Celiac disease is predisposed to become afflicted with an autoimmune neuropathy, comprising quantitating the amount of an antibody directed against a ganglioside in the subject using either of the instant methods, wherein the presence of a predefined amount of the antibody indicates that the subject is predisposed to become afflicted with an autoimmune neuropathy.

This invention further provides the instant method, wherein the antibody is directed against GM1.

This invention further provides the instant method, wherein the antibody is directed against GD1a.

BRIEF DESCRIPTION OF THE FIGURES

FIGURE 1: Analysis of patient sera with latex agglutination assay and ELISA.

FIGURE 2: Comparison of ELISA and latex agglutination assay in detection of anti-GM1 antibodies in sera of patients with MMN.

FIGURE 3: Latex agglutination assay in detection of anti-GM1 antibodies in sera of patients with MMN using latex particles coated with different ratios of GM1 to GD1a.

FIGURE 4: Analysis of patient sera with ELISA and latex agglutination assay.

FIGURE 5: Comparison of ELISA and latex agglutination assay for antiganglioside antibody-positive sera.

DETAILED DESCRIPTION OF THE INVENTION

This invention provides a method of detecting the presence of an antibody directed against a ganglioside in a subject comprising:

(a) contacting a liquid sample from the subject with the ganglioside, such ganglioside being affixed to at least two separate solid particles, under conditions permitting the antibody if present in the sample to form a complex with the ganglioside, which complex comprises such solid particles; and

(b) detecting the presence of any complex formed in step (a), wherein the presence of such complexes indicates the presence of the antibody in the subject.

Solid particles are generally constructed of unreactive material and are of consistent size, for example 0.3μm diameter latex polystyrene beads. Two separate particles having ganglioside there affixed can be bound by an antibody. In one embodiment ganglioside is covalently affixed to the microparticles. In a different embodiment the ganglioside is not covalently affixed to the microparticle. In one embodiment microparticles comprise polystyrene latex. In one embodiment the microparticles comprise carbonsol.

The subject includes, but is not limited to, a human, a primate, a mouse, a rat, a guinea pig or a rabbit. In a preferred embodiment the subject is a human.

In different embodiments the ganglioside is chosen from the group consisting of GM1, GM2, GM3, GD1, GD2, GD3, GD1a, GD1b, GT1b or GQ1b, where G = ganglioside. In another embodiment the ganglioside comprises total brain ganglioside extract. In a further embodiment the source of the extract is a bovid. In one embodiment the ganglioside comprises tissue ganglioside extract.

In one embodiment the antiganglioside antibody is an autoantibody. In differing embodiments the antiganglioside antibody is chosen from the group consisting of anti-GM1, anti-GM2, anti-GM3, anti-GD1, anti-GD2, anti-GD3, anti-GD1a, anti-GD1b, anti-GT1b or anti-GQ1b, where G = ganglioside, e.g. anti-GM1 is an antibody directed against GM-1. The terms 'antiganglioside antibody' and 'antibody directed against a ganglioside' are used interchangeably.

In one embodiment the sample is human sera. In differing embodiments the sample is chosen from the group consisting of plasma, saliva, tears, mucosal discharge, urine, peritoneal fluid, cerebrospinal fluid, lymphatic fluid, bone marrow, tissue, lymph nodes or culture media.

This invention also provides a method of detecting in a subject the presence of at least two different antibodies, each of which antibodies is directed against a different type of ganglioside comprising:

(a) contacting a liquid sample from the subject with one such type of ganglioside, such ganglioside being affixed to at least two separate solid particles, under conditions permitting the

antibody directed against said type of ganglioside if present in the sample to form a complex with the ganglioside, which complex comprises such solid particles;

(b) contacting such liquid sample with a different type of ganglioside, such different type of ganglioside being affixed to at least two separate solid particles, under conditions permitting the antibody directed against such different type of ganglioside if present in the sample to form a complex with such different type of ganglioside, which complex comprises such solid particles; and

(c) detecting the presence of any complex formed in step (b) and any complex formed in step (c), wherein the presence of complexes formed in both step (b) and step (c) indicates the presence in the subject of such different antibodies.

This invention further provides the instant method, wherein steps (a) and (b) are performed simultaneously.

This invention further provides the instant method, wherein the solid particles having affixed thereto said one such type of ganglioside are the same color and the solid particles having affixed thereto said different type of ganglioside are of a different color.

Solid particles are generally constructed of unreactive material and are of consistent size, for example 0.3μm diameter latex polystyrene beads. In one embodiment ganglioside is covalently affixed to the microparticles.

In a different embodiment the ganglioside is not covalently affixed to the microparticle. In one embodiment microparticles comprise polystyrene latex. In one embodiment the microparticles comprise carbonsol.

The subject includes, but is not limited to, a human, a primate, a mouse, a rat, a guinea pig or a rabbit. In a preferred embodiment the subject is a human.

In different embodiments the ganglioside is chosen from the group consisting of GM1, GM2, GM3, GD1, GD2, GD3, GD1a, GD1b, GT1b or GQ1b, where G = ganglioside. In another embodiment the ganglioside comprises total brain ganglioside extract. In a further embodiment the source of the extract is a bovid. In one embodiment the ganglioside comprises tissue ganglioside extract.

In one embodiment the antiganglioside antibody is an autoantibody. In differing embodiments the antiganglioside antibody is chosen from the group consisting of anti-GM1, anti-GM2, anti-GM3, anti-GD1, anti-GD2, anti-GD3, anti-GD1a, anti-GD1b, anti-GT1b or anti-GQ1b, where G = ganglioside as described hereinabove. The terms 'antiganglioside antibody' and 'antibody directed against a ganglioside' are used interchangeably.

In one embodiment the sample is human sera. In differing embodiments the sample is chosen from the group consisting of plasma, saliva, tears, mucosal discharge, urine, peritoneal fluid, cerebrospinal fluid, lymphatic fluid, bone marrow, tissue, lymph nodes or culture media.

This invention further provides the instant methods, wherein the antibody is directed against more than one ganglioside.

This invention further provides the instant methods, wherein the antibody is directed against one ganglioside.

This invention also provides a method of quantitating the amount of an antibody directed against a ganglioside present in a subject comprising:

(a) contacting a plurality of identical liquid samples from the subject with the ganglioside, each such sample comprising the ganglioside affixed to at least two separate solid particles, such particles having affixed thereto a predetermined amount of such ganglioside, wherein the predetermined amount used to contact each said sample is different, under conditions permitting the antibody if present in the sample to form a complex with the ganglioside, which complex comprises such solid particles; and

(b) detecting the presence in each such sample of any complex formed in step (a), and correlating such detection of complexes in each such sample with a predefined reference standard indicative of the amount of the antibody present in the subject so as to quantitate the amount of the antibody present in the subject.

This invention also provides a method of quantitating the amount of an antibody directed against a ganglioside present in a subject comprising:

(a) contacting a plurality of liquid samples from the subject with the ganglioside, each such sample being differently diluted and such ganglioside being affixed to at least two separate solid particles, such particles having affixed thereto a predetermined amount of such ganglioside, wherein the predetermined amount used to contact each said sample is the same, under conditions permitting the antibody if present in the sample to form a complex with the ganglioside, which complex comprises such solid particles; and

(b) detecting the presence in each such sample of any complex formed in step (a), and correlating such detection of complexes in each such sample with a predefined reference standard indicative of the amount of the antibody present in the subject so as to quantitate the amount of the antibody present in the subject.

Solid particles are generally constructed of unreactive material and are of consistent size, for example 0.3μm diameter latex polystyrene beads. In one embodiment ganglioside is covalently affixed to the microparticles. In a different embodiment the ganglioside is not covalently affixed to the microparticle. In one embodiment microparticles comprise polystyrene latex. In one embodiment the microparticles comprise carbonsol.

The subject includes, but is not limited to, a human, a primate, a mouse, a rat, a guinea pig or a rabbit. In a preferred embodiment the subject is a human.

In different embodiments the ganglioside is chosen from the group consisting of GM1, GM2, GM3, GD1, GD2, GD3, GD1a, GD1b, GT1b or GQ1b, where G = ganglioside. In another embodiment the ganglioside comprises total brain ganglioside extract. In a further embodiment the source of the extract is a bovid. In one embodiment the ganglioside comprises tissue ganglioside extract.

In one embodiment the antiganglioside antibody is an autoantibody. In differing embodiments the antiganglioside antibody is chosen from the group consisting of anti-GM1, anti-GM2, anti-GM3, anti-GD1, anti-GD2, anti-GD3, anti-GD1a, anti-GD1b, anti-GT1b or anti-GQ1b, where G = ganglioside. The terms 'antiganglioside antibody' and 'antibody directed against a ganglioside' are used interchangeably.

In one embodiment the sample is human sera. In differing embodiments the sample is chosen from the group consisting of plasma, saliva, tears, mucosal discharge, urine, peritoneal fluid, cerebrospinal fluid, lymphatic fluid, bone marrow, tissue, lymph nodes or culture media.

This invention further provides a method of diagnosing whether a subject has autoimmune neuropathy, comprising quantitating the amount of an antibody directed against a ganglioside in the subject using the instant methods, wherein the presence of a predefined amount of the antibody indicates that the subject is suffering from autoimmune neuropathy. In one embodiment the neuropathy is Guillain-Barré syndrome. In another embodiment the neuropathy is a Guillain-Barré syndrome variant. Examples

of Guillain-Barré syndrome variant include, but are not limited to, acute inflammatory demyelinating polyneuropathy, acute motor axonal neuropathy, Miller Fisher syndrome and acute motor and sensory axonal neuropathy. In one embodiment the neuropathy is a peripheral neuropathic disease. In one embodiment the neuropathy is a multifocal motor neuropathy.

This invention further provides a method of diagnosing whether a subject that has Celiac disease suffers from autoimmune neuropathy, comprising quantitating the amount of an antibody directed against a ganglioside in the subject using the instant method, wherein the presence of a predefined amount of the antibody indicates that the subject is suffering from autoimmune neuropathy. In one embodiment the antibody is directed against GM1. In one embodiment the antibody is directed against GD1a.

This invention further provides a method of determining if a subject is predisposed to become afflicted with an autoimmune neuropathy, comprising quantitating the amount of an antibody directed against a ganglioside in the subject using either of the instant methods, wherein the presence of a predefined amount of the antibody indicates that the subject is predisposed to become afflicted with an autoimmune neuropathy. In one embodiment the neuropathy is Guillain-Barré syndrome. In one embodiment the neuropathy is a Guillain-Barré syndrome variant. Examples of Guillain-Barré syndrome variant include, but are not limited to, acute inflammatory demyelinating polyneuropathy, acute motor axonal neuropathy, Miller Fisher syndrome and acute motor and sensory axonal

neuropathy. In one embodiment the neuropathy is multifocal motor neuropathy. In one embodiment the neuropathic disease is a peripheral neuropathic disease.

This invention further provides a method of determining if a subject with Celiac disease is predisposed to become afflicted with an autoimmune neuropathy, comprising quantitating the amount of an antibody directed against a ganglioside in the subject using either of the instant methods, wherein the presence of a predefined amount of the antibody indicates that the subject is predisposed to become afflicted with an autoimmune neuropathy. In one embodiment the antibody is directed against GM1. In one embodiment the antibody is directed against GD1a. In one embodiment the subject is known to have Celiac disease. In another embodiment the subject is not known to have Celiac disease.

This invention will be better understood by reference to the Experimental Details which follow, but those skilled in the art will readily appreciate that the specific experiments detailed are only illustrative of the invention as described more fully in the claims which follow thereafter.

EXPERIMENTAL DETAILS

First Series of Experiments

Materials and Methods

Serum Samples

Serum samples were obtained from 29 patients; eight with multifocal motor neuropathy (MMN), ten with chronic inflammatory demyelinating polyneuropathy (CIDP), six with amyotrophic lateral sclerosis (ALS), four with demyelinating neuropathy associated with anti-myelin-associated glycoprotein (anti-MAG) antibodies, and one with Miller Fisher syndrome (MFS). In addition, sera from five normal subjects were evaluated as controls. All patient sera were prepared, aliquoted, and stored at -20 °C.

Preparation of Latex Particles

Latex beads were coated with GM1 ganglioside by passive adsorption. A 400 mg/mL solution of GM1 ganglioside (Sigma Chemicals, St. Louis, MO) was prepared by combining 40 mL of a 5 mg/mL stock solution of GM1 in methanol with 210 mL of H_2O and 250 mL of 100 mM 2-(N-morpholino)ethanesulfonic acid (MES) buffer (pH 6.1). A 1% suspension of 0.3 m blue polystyrene latex particles (Seradyn Particle Technology, Indianapolis, IN) was prepared from the 2.5% stock suspension by adding H_2O. Adsorption of GM1 to the beads was initiated by addition of microparticle suspension to the ganglioside solution, followed by gentle stirring for 4 hours at room

temperature. The suspension was then incubated for 72 hours at 4 °C. The particles were washed twice with a solution of 1% BSA in 25 mM MES buffer (pH 6.1) by centrifugation at 9,800 x *g* and 4 °C, and resuspended in the same solution. The coated beads were incubated for 48 hours at 4 °C before use. Control latex particles were prepared by coating them with GD1a ganglioside (Sigma Chemicals, St. Louis, MO) in place of GM1, following the same procedure.

To determine whether titers of anti-GM1 antibodies could be quantified by testing for reactivity with beads containing decreasing concentrations of GM1, sera were tested for agglutination using beads that were coated with varying concentrations of GM1 and GD1a. Preparation of the latex particles was the same as described for GM1, with the difference that increasing quantities of GD1a were used to replace GM1, effectively lowering the concentration of GM1 coated. The following concentrations of GM1 were examined: 100% GM1, 50% GM1, 12% GM1, 6% GM1, 1.5% GM1, 0.75% GM1, and 0% GM1.

Agglutination Reaction

On a 3-ring glass slide (Cel-Line, Newfield, NJ), 4.5 mL aliquots of serum were placed. To each ring, 4.5 mL of the coated latex particles was added and mixed thoroughly with a plastic applicator. The slide was rocked gently for 30 to 40 seconds. Positive agglutination, characterized by blue clumps of beads, indicated the presence of anti-GM1 antibodies. Particle agglutination was more easily visualized when using colored latex beads

instead of white beads. Strong results were clearly visible with the naked eye. Weak results could be visualized by holding the slide to a light source and observing for agglutination from underneath. To minimize inter-operator variability, all results were confirmed using a microscope (x 40 magnification). In the absence of agglutination, the reaction was considered to be negative. If agglutination were present, it was scored from 1 to 3 according to the degree of agglutination, where 1 denotes weak agglutination and 3 strong agglutination.

Enzyme-Linked Immunosorbent Assay (ELISA)

The presence of anti-GM1 IgM in sera was also measured by the commonly used enzyme-linked immunosorbent assay, following previously described procedure (11), with minor modification. Wells in 96-well round-bottom polystyrene microtiter plates (Becton Dickinson, Franklin Lakes, NJ) were coated with 0.5 mg of GM1 in 100 mL of methanol. After evaporation of the methanol, the wells were blocked by incubation with 300 mL of 1% bovine serum albumin (BSA) in 10 mM phosphate-buffered saline (154 mM NaCl, pH 7.4) (PBS) for 4 hours at 4 °C, and 100 mL of BSA/PBS-diluted patient or control serum was added to the wells. Wells coated with BSA instead of serum served as control. The plates were incubated overnight at 4 °C and then washed with the BSA/PBS solution. Antibody binding was detected by the addition of 100 mL peroxidase-conjugated goat anti-human IgM secondary antibody (ICN Biomedicals, Costa Mesa, CA) after 1:1000 dilution in BSA/PBS solution (a final concentration of 2.14 mg/mL) to each well, and

incubation for 2 hours at 4 °C. Plates were then washed and 100 mL of developing solution comprised of 27 mM citric acid, 50 mM Na_2HPO_4, 5.5 mM o-phenylenediamine, and 0.01% H_2O_2 (pH 5-5.5) was added to each well. The plates were incubated at room temperature for 30 minutes before measuring absorbance at 450 nm. The titer for each specimen was assigned as the highest dilution in which the absorbance reading was 0.1 units greater than in the corresponding BSA-coated wells. Sera with titers of 800 or lower were considered to be negative for the presence of clinically significant amounts of anti-GM1 antibodies, as such titers are also seen in normal subjects (10).

Results

Sera from a total of 34 individuals were examined for anti-GM1 antibodies by both the agglutination assay and ELISA. Of the eight sera examined from MMN patients, six tested positive for anti-GM1 antibodies by the latex agglutination assay. A 11 sera from patients with CIDP, ALS, demyelinating neuropathy associated with anti-MAG antibodies, and MFS, as well as those from normal subjects were found to be negative (FIGURE 1). All specimens were tested on at least three different occasions. The assay proved to have a high reproducibility as repeated tests on each serum gave identical results, with the rankings remaining the same.

Altering the concentration of coated GM1 antigen led to differences in reactivity with each serum. Undiluted sera with higher titers of anti-GM1 antibodies, as determined by ELISA, caused agglutination of

microparticles coated with lower concentrations of antigen. The new agglutination assay was designed in such a manner as to give positive results only when testing sera with clinically significant titers of anti-GM1 antibodies. The sensitivity of the assay system was mainly dependent on the antigen concentration, that is the concentration of the coated GM1 ganglioside. That concentration was therefore adjusted to yield positive agglutination results with patient sera exhibiting anti-GM1 antibody titers of 800 or above, as measured in the ELISA system. Optimal results were obtained with incubation of a 1% suspension of 0.3 m latex beads with a 400 mg/mL solution of GM1.

The agglutination assay exhibited equally good or better sensitivity when compared to the ELISA system. It gave positive results in all 5 of the 8 patients with MMN and elevated anti-GM1 antibodies as determined by ELISA, with titers ranging between 1,600 and 100,000 (FIGURE 2). One other patient with MMN was positive by the agglutination assay but negative by ELISA, with a titer of 800. The two remaining patients with MMN were negative for anti-GM1 antibodies by both the agglutination and ELISA systems.

The agglutination assay appeared to be highly specific for patients with MMN, with none of the control patients or normal subjects exhibiting positive results. Four specimens with elevated levels of serum IgM and increased titers of anti-MAG antibodies, as well as a specimen from a patient with Miller Fisher syndrome (MFS) and antibodies against GQ1b ganglioside, tested negative for

reactivity to GM1 with the agglutination assay.

Four of the samples that exhibited reactivity to GM1 ganglioside in the agglutination assay were also tested for reactivity with latex particles coated with decreasing concentrations of GM1, in which GD1a was substituted (FIGURE 3). None of the sera caused agglutination with particles coated with 100% GD1a, thus confirming the specificity of the GM1 reaction. On the other hand, all four sera yielded positive results with particles coated with less than 100% GM1; the higher the titer of anti-GM1 antibodies, the lower the concentration of the GM1 antigen that was required to produce agglutination. The serum with the highest concentration of anti-GM1 antibodies, having a titer of 100,000 by ELISA, reacted with beads that were coated with as little as 1.5% GM1.

DISCUSSION

A novel latex agglutination assay was developed for detection of serum anti-GM1 antibodies. The assay detects a functional antibody-antigen interaction that results in agglutination and compares favorably to the ELISA system in sensitivity and specificity. Additional advantages of the new assay include substantial reduction in the cost and time required for performing the test. Unlike the ELISA, which takes two days to perform and requires a plate reader, the agglutination assay is completed in minutes and requires no special instruments.

The agglutination assay can be readily used to rapidly

screen sera for the presence of anti-GM1 antibodies. In light of the fact that a large number of sera are negative for the presence of anti-GM1 antibodies, the assay aids in screening out negative serum samples. If information on antibody titer is desired, reactive sera can then be tested using the ELISA system, which measures antibody binding at increasing serum dilutions, or by the agglutination assay, which tests for reactivity using microparticles coated with decreasing antigen concentrations.

In addition to testing for antibodies to isolated glycolipids such as GM1, the agglutination assay could be useful in detecting antibody reactivities to one or more antigens in a mixture of glycolipids coated onto the latex particles. This could be used in the form of sensitive assays for detection of antibodies that react with shared epitopes on two or more glycolipids (14), or that recognize conformational epitopes that result from the interaction of two or more neighboring glycolipids (15). It could also be particularly useful in testing for the presence of antibodies directed against previously unrecognized antigenic glycolipids in other immune-mediated disorders.

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Second Series of Experiments

MATERIALS AND METHODS

Serum samples

Serum samples were obtained from 45 patients: twelve with multifocal motor neuropathy (MMN), thirteen with Guillain-Barré syndrome (GBS), ten with chronic inflammatory demyelinating polyneuropathy (CIDP), six with amyotrophic lateral sclerosis (ALS), and four with demyelinating neuropathy associated with anti-myelin-associated glycoprotein (anti-MAG) antibodies. Criteria used for patient classification have been described before (11-14). In addition, serum samples from ten normal subjects were evaluated as controls. All patient sera were stored at -20 °C.

Preparation of Latex Particles

Preparation of the microparticles was optimized particularly with regard to the amount of antigen coated on the surface of the particles, and the type of medium employed in the initiation of the reaction, such that normal sera would test negative in the final assay. Latex beads were coated with a total ganglioside preparation (Ca^{2+} salt) by passive adsorption. A 2 mg/mL solution of gangliosides (Sigma Chemicals, St. Louis, MO) was prepared by combining 105 mL of a 4.76 mg/mL stock solution of gangliosides in H_2O with 20 mL of methanol and 125 mL of 100 mM 2-(N-morpholino)ethanesulfonic acid (MES) buffer (pH 6.1). A 1% suspension of 0.3 m blue polystyrene latex particles (Seradyn Particle Technology, Indianapolis, IN) was prepared from the 2.5% stock suspension by adding H_2O. Adsorption of gangliosides to the beads was initiated by addition of 125 mL of microparticle suspension to the ganglioside solution, followed by gentle stirring for 4 hrs at room temperature. The suspension was then incubated for 72 hours at 4 °C. The particles were washed twice with a solution of 1% bovine serum albumin (BSA) in 25 mM MES buffer (pH 6.1) by centrifugation at 9,800 x g and 4 °C, and resuspended in the same solution. The coated beads were incubated for 48 hrs at 4 °C before use.

Agglutination Reaction

On a 3-ring glass slide (Cel-Line, Newfield, NJ), 5 mL aliquots of serum were placed. To each ring, 5 mL of the coated latex beads was added and mixed thoroughly with a plastic applicator. The slide was rocked gently for 30

to 40 seconds. Positive agglutination, characterized by blue clumps of beads, indicated the presence of anti-ganglioside antibodies. Colored latex beads were used instead of white beads because of the ease with which positive agglutination results could be visualized. Strong results were clearly visible with the naked eye. Weak results could be visualized by holding the slide to a light source, and observing for agglutination from underneath. In order to minimize inter-operator variability, all results were confirmed using a microscope (x 40 magnification). Results were scored from 1 to 3 according to the degree of agglutination, while in the absence of agglutination, the reaction was considered to be negative.

Enzyme-linked Immunosorbent Assay (ELISA)

The presence of antibodies directed against GM1 and GQ1b in sera was determined by the enzyme-linked immunosorbent assay, following previously described procedure (15), with minor modification. Wells in 96-well round-bottom polystyrene microtiter plates (Becton Dickinson, Franklin Lakes, NJ) were coated with 0.5 mg of the individual gangliosides (Sigma Chemicals, St. Louis, MO) in 100 mL of methanol. Wells to which only methanol was added served as controls. After evaporation of the methanol, all wells were blocked by incubation with 300 mL of 1% BSA in 10 mM phosphate-buffered saline (154 mM NaCl, pH 7.4) (PBS) for 4 hours at 4 °C. The plates were incubated overnight at 4 °C, and then washed with the BSA/PBS solution. This was followed by the addition of 100 mL of peroxidase-conjugated goat anti-human IgM or IgG

secondary antibody (ICN Biomedicals, Costa Mesa, CA) after 1:1000 and 1:800 dilution respectively in BSA/PBS solution (a final concentration of 2.14 mg/mL for both antibodies) to each well, and incubation for 2 hours at 4 °C. Plates were then washed as before and 100 mL of developing solution comprised of 27 mM citric acid, 50 mM Na_2HPO_4, 5.5 mM o-phenylenediamine, and 0.01% H_2O_2 (pH 5-5.5) was added to each well. The plates were incubated at room temperature for 30 min, before measuring absorbance at 450 nm. The titer for each specimen was assigned as the highest dilution in which the absorbance reading was 0.1 units greater than in the corresponding control well. Sera with titers of 800 or less were considered to be negative for the presence of clinically significant amounts of antibodies against GM1, as such titers are also seen in normal subjects (9, 10). Similarly, only sera with titers of 100 and above were considered positive for anti-GQ1b antibodies.

Results

Sera from a total of 55 individuals were examined for anti-ganglioside antibodies by the agglutination immunoassay and ELISA. Of the twelve sera from MMN patients, eight were positive by both the agglutination assay (for anti-ganglioside antibodies), and the ELISA (for anti-GM1 antibodies). Of the thirteen sera from GBS patients, seven were positive for anti-ganglioside antibodies by the agglutination assay, while only four of these were positive for antibodies directed against GM1 or GQ1b by the ELISA system. All sera from patients with CIDP, ALS, and demyelinating neuropathy associated with

MAG antibodies, in addition to those from normal subjects were found to be negative (FIGURE 4). The new assay demonstrated high reproducibility as repeated tests on sera in a period of one week gave identical results, with the rankings staying the same.

With regard to sera from patients with MMN where the antibody is directed against the GM1 ganglioside, the agglutination assay showed equally good sensitivity when compared to the ELISA system. It gave positive results in all 8 of the 12 patients with MMN and elevated titers of anti-GM1 antibodies as determined by ELISA, with titers ranging between 1,600 and 102,400 (FIGURE 5). All serum samples from MMN patients with titers of 800 or less tested negative by the agglutination assay.

In analysis of sera from GBS patients, where the presence of several different anti-ganglioside antibody species have been reported, more patient sera were positive by the agglutination assay than the ELISA system. The two sera with elevated levels of IgG anti-GM1 antibodies and the two with elevated levels of IgG anti-GQ1b antibodies, with titers ranging from 100 to 25,600, as determined by ELISA, also tested positive with the agglutination assay. In addition, three other sera, which were found to be negative for antibodies against GM1 and GQ1b by ELISA, were positive for anti-ganglioside antibodies by the new agglutination assay. The remaining six serum samples were negative by both assays.

With the limited number of samples examined, the new assay demonstrated high specificity for patients with MMN and GBS, as none of the other patients or normal subjects

exhibited positive results. Four sera with elevated levels of serum IgM and increased titers of anti-MAG antibodies tested negative for reactivity to gangliosides with the agglutination assay. Solutions of nonspecific human IgM and IgG in MES buffer (1mg/mL) also yielded negative results when tested with the assay.

Multiple antibody detection

We tested sera for antibodies against multiple gangliosides in a single agglutination assay.

Materials and Methods

Sera from 256 patients with acute or chronic neuropathies, 6 patients with amyotrophic lateral sclerosis (ALS), and 10 normal subjects were tested for anti-ganglioside antibodies by the agglutination assay. Polystyrene microparticles were coated with a total ganglioside extract from bovine brain. When combined with serum, agglutination of microparticles signaled the presence of anti-ganglioside antibodies. Sera found to be positive by the agglutination assay were also tested by ELISA for IgM, IgG, and IgA antibodies to GM1, GM2, GD1a, GD1b, GQ1b, and GT1b gangliosides. Prior to the study, all sera were tested for anti-GM1 antibodies by ELISA.

Results

In the acute neuropathy group, 6 of 11 patients with Guillain-Barré Syndrome (GBS), 2 of 2 with Miller-Fisher

Syndrome (MFS), and 1 with bilateral facial palsy were reactive by the ganglioside agglutination assay. When tested by ELISA, of the 6 GBS sera, 1 was positive for GM1, GM2, and GD1b, 1 for GM1 and GD1b, and 1 for GD1a alone, while 3 were unreactive. Sera from the 3 patients with MFS or bilateral facial palsy all reacted with GQ1b. In the chronic neuropathy group, 12 of 14 patients with multifocal motor neuropathy (MMN), and 5 of 214 patients with other types of neuropathy were positive by the new assay. In the ELISA system, of the 12 reactive MMN sera, 4 were positive for GM1 and GD1b, 3 for GM1 alone, 3 for GM1 and GM2, plus GD1a or GD1b, 1 for GM1, GD1b, and GQ1b, and 1 for GQ1b alone. Of the other 5 reactive sera, the ELISA system demonstrated binding to GM1 and GD1b in one, to GM1 alone in another, and no reactivity in 3. All 16 control sera were negative by the agglutination assay. All sera that were previously known to be positive for GM1 by the ELISA system were also positive by the new assay.

Discussion

These results show that the ganglioside agglutination system provides a rapid method for detecting antibodies to multiple gangliosides in a single assay. Sera that are positive by the agglutination assay, but negative by ELISA for the individual gangliosides tested, may recognize minor gangliosides or conformational epitopes which are not available in the ELISA system. The assay is useful for screening patients with suspected autoimmune neuropathies, particularly in situations where quick diagnosis is desired, as in the Guillain-Barré syndrome.

Also diagnosis of other autoimmune diseases presenting antiganglioside antibodies may be accelerated using this assay.

Titering by Sera Dilution

Instead of titering with antigens, titers can alternatively be performed using sera dilutions.

Materials and Methods

Such experiments were performed with the following agglutination reaction: On a 3-ring glass slide (Cel-Line, Newfield, NJ), 5 mL aliquots of serum were placed. To each ring, 5 mL of the coated beads was added and mixed with a plastic applicator. The slide was rocked gently for 30 seconds. Positive agglutination, characterized by blue clumps of beads, indicated the presence of anti-ganglioside antibodies. Results were confirmed using a light microscope (x 40 magnification) and scored from 1 to 3 according to the degree of agglutination, where 1 denoted weak agglutination and 3 strong agglutination. In the absence of agglutination, the reaction was considered to be negative. Titration of sera was done only if the screening test was positive. Serial dilutions of sera were prepared in 10 mM phosphate-buffered saline (154 mM NaCl, pH 7.4) (PBS), in multiples of three. The titer for each specimen was assigned as the highest dilution in which the assigned score for the degree of agglutination was 1. All results were confirmed twice to reduce inter-operator

variability.

Results

Sera was drawn from 112 individuals in this study. Sera were obtained from 40 patients with Guillain-Barré syndrome (GBS). Twenty eight of those in the GBS group were classified as acute inflammatory demyelinating polyneuropathy (AIDP), 7 as acute motor axonal neuropathy (AMAN), 1 as acute motor and sensory axonal neuropathy (AMSAN), and 4 as Miller Fisher syndrome (MFS). In addition, serum samples from 6 patients with amyotrophic lateral sclerosis (ALS), 20 patients with multiple sclerosis (MS), and 46 normal subjects were evaluated as controls. Standard ELISA tests were also performed.

Twenty one of the GBS patients (53%) were positive for anti-ganglioside antibodies by the agglutination immunoassay. Antibody titers ranged from 1 to 48. In comparison, 17 GBS patients (43%) showed elevated antibody levels when tested by ELISA for IgM and IgG antibodies against GM1, GM2, GD1a, GD1b, GT1b, and GQ1b, with titers ranging from 100 to 25,600. All samples that were positive by ELISA were also positive by the agglutination assay. No binding to GT1b was observed in any of the sera. For samples positive by both assays, antibody titers determined by sera dilution found with the agglutination assay showed correlation with those found by ELISA in most cases. All samples from patients with ALS or MS, or from normal subjects, were found to be negative by both assays. Among the 40 GBS sera, 12 of 28 from AIDP patients (43%), 5 of 7 from AMAN patients

(71%), 3 of 4 from MFS patients (75%), and the one from the AMSAN patient, tested positive for anti-ganglioside antibodies by the agglutination assay.

Discussion

Measurement of serum anti-ganglioside autoantibody levels is increasingly used in the evaluation of patients with immune-mediated neuropathies. The currently available ELISA systems, however, are relatively time consuming and costly, and their use is limited due to issues of methodology, laboratory variability, and interpretation (16-20). Furthermore, in using these methods, testing against only a few standard gangliosides may miss some of the reactivities, whereas testing against every putative ganglioside antigen is inefficient and not always possible. In this study, a simple and quick agglutination assay capable of detecting a functional antibody-antigen interaction is described.

In patients with MMN, where the target antigen is the GM1 ganglioside, the new agglutination assay and ELISA yielded identical results. The degree of agglutination, however, was not found to correspond well to antibody titers as determined by ELISA, possibly due to differences in assay conditions. In contrast to the ELISA system, which measures binding of highly diluted serum at 4 °C, the agglutination assay is performed under more physiologic elements of temperature and serum concentration, and measures a more functional interaction. The agglutination assay may thus better represent the antibody-antigen interaction that takes

place in the human body.

In patients with GBS, the higher positivity rate for the agglutination assay (7/13) in comparison with ELISA (4/13) may be explained by the fact that the new assay detects the presence of all antiganglioside antibodies present in the serum, regardless of specificity or isotype. Sera from patients with GBS may cross react with or have antibodies to multiple gangliosides, including minor ones (21-23), and although most of the antibodies are IgG, antibodies of the IgM and IgA isotype have also been reported (24). We tested the sera against GM1 and GQ1b, which are the most common antigens described, but testing for all other gangliosides was beyond the scope of this study.

The new assay offers several advantages to the currently used ELISA system. It can detect the presence of antibodies to different gangliosides, while requiring only a few minutes to complete, and being more economical. It would be particularly useful in situations where rapid diagnosis and therapy are essential, as in the Guillain-Barré syndrome.

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Third Series of Experiments

Celiac disease is an autoimmune gastrointestinal disorder, mediated by antibodies and T cells, which is provoked by ingestion of gluten proteins present in wheat, barley, and rye. It has been associated with peripheral neuropathy as well other neurological disorders. We analyzed sera from 20 patients with celiac disease for the presence of antiganglioside antibodies by the ganglioside agglutination immunoassay using microparticles coated with a total extract of bovine brain gangliosides. Controls can be taken from patients without celiac disease. Of the 20 sera tested, 5 were reactive by the agglutination assay. Of these 5 reactive sera, 4 were known to have peripheral neuropathy. When tested by ELISA for IgG, IgM, and IgA antibodies against GMI and GDIa gangliosides, one serum was positive for IgG antibodies against GMI and GDIa, one for IgG antibodies to GMI, and a third for IgG antibodies to GDIa. The two sera reactive by agglutination and negative by ELISA probably have antibodies to other, possibly minor gangliosides, or to conformation epitopes not detected by ELISA. The neuropathy associated with celiac disease appears to be associated with antiganglioside antibodies, which may contribute to the disease. The presence of IgG reactivity furthermore implicates a T cell-mediated response to ganglioside antigens.

What is claimed is:

1. A method of detecting the presence of an antibody directed against a ganglioside in a subject comprising:

(a) contacting a liquid sample from the subject with the ganglioside, such ganglioside being affixed to at least two separate solid particles, under conditions permitting the antibody if present in the sample to form a complex with the ganglioside, which complex comprises such solid particles; and

(b) detecting the presence of any complex formed in step (a), wherein the presence of such complexes indicates the presence of the antibody in the subject.

2. A method of detecting in a subject the presence of at least two different antibodies, each of which antibodies is directed against a different type of ganglioside comprising:

(a) contacting a liquid sample from the subject with one such type of ganglioside, such

ganglioside being affixed to at least two separate solid particles, under conditions permitting the antibody directed against said type of ganglioside if present in the sample to form a complex with the ganglioside, which complex comprises such solid particles;

(b) contacting such liquid sample with a different type of ganglioside, such different type of ganglioside being affixed to at least two separate solid particles, under conditions permitting the antibody directed against such different type of ganglioside if present in the sample to form a complex with such different type of ganglioside, which complex comprises such solid particles; and

(c) detecting the presence of any complex formed in step (b) and any complex formed in step (c), wherein the presence of complexes formed in both step (b) and step (c) indicates the presence in the subject of such different antibodies.

3. The method of claim 2, wherein steps (a) and (b) are performed simultaneously.

4. The method of claim 2, wherein the solid particles having affixed thereto said one such type of ganglioside are the same color and the solid particles having affixed thereto said different type of ganglioside are of a different color.

5. The method of claim 1 or 2, wherein the antibody is directed against more than one ganglioside.

6. The method of claim 1 or 2, wherein the antibody is directed against one ganglioside.

7. A method of quantitating the amount of an antibody directed against a ganglioside present in a subject comprising:

(a) contacting a plurality of identical liquid samples from the subject with the ganglioside, each such sample comprising the ganglioside affixed to at least two separate solid particles, such particles having affixed thereto a predetermined amount of such ganglioside, wherein the predetermined amount used to contact each said sample is different,

under conditions permitting the antibody if present in the sample to form a complex with the ganglioside, which complex comprises such solid particles; and

(b) detecting the presence in each such sample of any complex formed in step (a), and correlating such detection of complexes in each such sample with a predefined reference standard indicative of the amount of the antibody present in the subject so as to quantitate the amount of the antibody present in the subject.

8. A method of quantitating the amount of an antibody directed against a ganglioside present in a subject comprising:

(a) contacting a plurality of liquid samples from the subject with the ganglioside, each such sample being differently diluted and such ganglioside being affixed to at least two separate solid particles, such particles having affixed thereto a predetermined amount of such ganglioside, wherein the predetermined amount used to contact each said sample is the same, under conditions permitting the antibody if

present in the sample to form a complex with the ganglioside, which complex comprises such solid particles; and

(b) detecting the presence in each such sample of any complex formed in step (a), and correlating such detection of complexes in each such sample with a predefined reference standard indicative of the amount of the antibody present in the subject so as to quantitate the amount of the antibody present in the subject.

9. The method of claim 1, 2, 7 or 8, wherein the liquid sample is human sera.

10. The method of claim 1, 2, 7 or 8, wherein the liquid sample is chosen from the group consisting of plasma, saliva, tears, mucosal discharge, urine, peritoneal fluid, cerebrospinal fluid, lymphatic fluid, bone marrow, tissue, lymph nodes or culture media.

11. The method of claim 1, 2, 7 or 8, wherein the solid particles comprise polystyrene latex.

12. The method of claim 1, 2, 7 or 8, wherein the solid particles comprise carbonsol.

13. The method of claim 1, 2, 7 or 8, wherein the ganglioside is covalently affixed to the solid particles.

14. The method of claim 1, 2, 7 or 8, wherein the ganglioside is chosen from the group consisting of GM1, GM2, GM3, GD1, GD2, GD3, GD1a, GD1b, GT1b or GQ1b.

15. The method of claim 1, 2, 7 or 8, wherein the ganglioside comprises total brain ganglioside extract.

16. The method of claim 15, wherein the source of the extract is a bovid.

17. The method of claim 1, 2, 7 or 8, wherein the ganglioside comprises tissue ganglioside extract.

18. The method of claim 1, 2, 7 or 8, wherein the antiganglioside antibody is an autoantibody.

19. The method of claim 1, 2, 7 or 8, wherein the antiganglioside antibody is chosen from the group consisting of anti-GM1, anti-GM2, anti-GM3, anti-GD1, anti-GD2, anti-GD3, anti-GD1a, anti-GD1b, anti-GT1b or anti-GQ1b.

20. A method of diagnosing whether a subject has autoimmune neuropathy, comprising quantitating the amount of an antibody directed against a ganglioside in the subject using the method of claim 7 or 8, wherein the presence of a predefined amount of the antibody indicates that the subject is suffering from autoimmune neuropathy.

21. A method of diagnosing whether a subject that has Celiac disease suffers from autoimmune neuropathy, comprising quantitating the amount of an antibody directed against a ganglioside in the subject using the method of claim 7 or 8, wherein the presence of a predefined amount of the antibody indicates that the subject is suffering from autoimmune neuropathy.

22. The method of claim 21, wherein the antibody is

directed against GM1.

23. The method of claim 21, wherein the antibody is directed against GD1a.

24. The method of claim 19, wherein the neuropathy is Guillain-Barré syndrome.

25. The method of claim 19, wherein the neuropathy is a Guillain-Barré syndrome variant.

26. The method of claim 19, wherein the neuropathy is a peripheral neuropathic disease.

27. The method of claim 19, wherein the neuropathy is a multifocal motor neuropathy.

28. A method of determining if a subject is predisposed to become afflicted with an autoimmune neuropathy, comprising quantitating the amount of an antibody directed against a ganglioside in the subject using the method of claim 7 or 8, wherein the presence of a predefined amount of the antibody indicates that the subject is predisposed to become afflicted with

an autoimmune neuropathy.

29. The method of claim 28, wherein the neuropathy is Guillain-Barré syndrome.

30. The method of claim 28, wherein the neuropathy is a Guillain-Barré syndrome variant.

31. The method of claim 28, wherein the neuropathy is a peripheral neuropathic disease.

32. The method of claim 28, wherein the neuropathy is a multifocal motor neuropathy.

33. A method of determining if a subject with Celiac disease is predisposed to become afflicted with an autoimmune neuropathy, comprising quantitating the amount of an antibody directed against a ganglioside in the subject using the method of claim 7 or 8, wherein the presence of a predefined amount of the antibody indicates that the subject is predisposed to become afflicted with an autoimmune neuropathy.

34. The method of claim 33, wherein the antibody is

directed against GM1.

35. The method of claim 33, wherein the antibody is directed against GD1a.

FIGURE 1

Analysis of Patient Sera with Latex Agglutination Assay ELSIA

Group	Number of serum samples	Number positive by latex agglutination assay	Number positive by ELISA
MMN	8	6	5
CIDP	10	0	0
ALS	6	0	0
Anti-MAG Neuropathy	4	0	0
MFS	1	0	0
Normal	5	0	0

FIGURE 2

Comparison of ELSIA and LATEX Agglutination Assay in Detection of Anti--GM1 Antibodies in Sera of Patients with MMN

Patient No.	Anti-GM1 IgM Titer (ELISA)[1]	Latex Agglutination Assay[2]
1	100,000	3
2	3,200	3
3	50,000	3
4	<800	Negative
5	800	1
6	1,600	2
7	<800	Negative
8	6,400	3

[1]Titer for each specimen was assigned as the highest dilution in which the absorbance reading was 0.1 units greater than in the corresponding BSA coated wells.
[2]Results were scored from 1 to 3 according to the degree of agglutination.

FIGURE 3

Latex Agglutination Assay in Detection of Anti--GM1 Antibodies in Sera of Patients with MMN. Using Latex Particles Coated with Different Ratios of GM1 to GD 1a

Patient No.	Anti-GM1 IgM Titer (ELISA)[1]	Latex Agglutination Assay[2]						
		A	B	C	D	E	F	G
1	100,000	3	2	2	2	1	Neg.	Neg.
3	50,000	3	2	1	Neg.	Neg.	Neg.	Neg.
6	1,600	2	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.
8	6,400	3	1	Neg.	Neg.	Neg.	Neg.	Neg.

[1]Titer for each specimen was assigned as the highest dilution in which the absorbance reading was 0.1 units greater than in the corresponding BSA coated wells.

[2]A: 100% GM1, 0% GD1a; B: 50% GM1, 50% GD1a; C: 12% GM1, 88% GD1a; D: 6% GM1, 94% GD1a; E: 1.5% GM1, 98.5% GD1a; F: 0.75% GM1, 99.25% GD1a; G: 0% GM1, 100% GD1a.

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FIGURE4

Analysis of patient sera with ELSIA and latex agglutination assay

Group	Number of Specimens	Number positive by ELISA	Number positive by agglutination assay
MMN	12	8	8
CIDP	10	0	0
ALS	6	0	0
Anti-MAG Neuropathy	4	0	0
GBS	13	4	7
Normal	10	0	0

FIGURE 5

Comparison of ELISA and latex agglutination assay for antiganglioside antibody-positive sera.

Patient No.	Group	ELISA Antiganglioside Antibody Titer[a] GM1	GQ1b	Agglutination Assay[b]
1	MMN	102,400	-	3
2	MMN	3,200	-	2
3	MMN	51,200	-	2
7	MMN	1,600	-	2
9	MMN	6,400	-	1
10	MMN	12,800	-	2
11	MMN	3,200	-	1
12	MMN	25,600	-	2
30	GBS	-	-	2
31	GBS	-	-	1
33	GBS	6,400	-	3
37	GBS	-	-	2
39	GBS	25,600	-	3
40	GBS(MFS variant)	-	400	2
41	GBS(MFS variant)	-	100	2

[a] Titer for each specimen was assigned as the highest dilution in which the absorbance reading was 0.1 units greater than in the corresponding control wells.

[b] Results were scored from 1 to 3 according to the degree of agglutination.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US01/26708

A. CLASSIFICATION OF SUBJECT MATTER

IPC(7) :G01N 33/53, 33/543, 33/545, 33/546, 33/564

US CL :435/7.21, 7.23; 436/506, 518, 523, 528, 531, 534

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 435/7.2, 7.21, 7.23, 7.25, 7.92; 436/506, 518, 519, 520, 523, 524, 528, 531, 534

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

DIALOG, EAST

search terms: glycolipid, gm, ganglioside, latex, polystyrene, autoantibod?, agglutinat?, aggregat?

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	UHLIG et al. Monoclonal Autoantibodies Derived from Multiple Sclerosis Patients and Control Persons and their Reactivities with Antigens of the Central Nervous System. Autoimmunity. 1989, Vol. 5, pages 87-99, entire document, especially pages 91-95 and Fig. 2.	1-35
Y	US 5,443,952 A (PESTRONK) 22 August 1995, entire document, especially cols. 7-10 and Fig. 7.	1-35
Y	DWYER et al. Cholera Toxin Mediated Agglutination of Ganglioside GM1 Containing Phospholipid Vesicles and GM1-Coated Polystyrene Spheres. Biochemistry. 1982, Vol. 21, pages 3231-3234, entire document.	1-35

[X] Further documents are listed in the continuation of Box C. [ ] See patent family annex.

* Special categories of cited documents:	
"A" document defining the general state of the art which is not considered to be of particular relevance	
"E" earlier document published on or after the international filing date	
"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	
"O" document referring to an oral disclosure, use, exhibition or other means	
"P" document published prior to the international filing date but later than the priority date claimed	
"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention	
"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone	
"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art	
"&" document member of the same patent family	

Date of the actual completion of the international search 21 DECEMBER 2001	Date of mailing of the international search report 25 JAN 2002
Name and mailing address of the ISA/US Commissioner of Patents and Trademarks Box PCT Washington, D.C. 20231 Facsimile No. (703) 305-3230	Authorized officer Felicia D. Roberts for JAMES L. GRUN, PH.D. Telephone No. (703) 308-0196

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INTERNATIONAL SEARCH REPORT

International application No.
PCT/US01/26708

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	UEMURA et al. The Reactivities of Human Erythrocyte Autoantibodies Anti-Pr2, Anti-Gd, F1 and Sa with Gangliosides in a Chromatogram Binding Assay. Biochemical Journal. 1984, Vol. 219, pages 865-874, especially Table 1.	1-35
Y	RAVINDRANATHS et al. Human Melanoma Antigen O-Acetylated Ganglioside GD3 is Recognized by Cancer antennarius Lectin. Journal of Biological Chemistry. 05 February 1988, Vol. 263, No. 4, pages 2079-2086, especially page 2080, col. 2.	1-35
A	YI et al. Rapid GM1 Ganglioside Latex Agglutination Slide Test for Cholera Toxin. Journal of Rapid Method and Automation in Microbiology. December 1992, Vol. 1, No. 3, pages 205-209.	1-35
A	VAISHNAVI et al. Field Utility of Phenolic Glycolipid Coated Latex Agglutination Test for Rapid Detection of Bacilliferous Leprosy Cases. Journal of Hygiene, Epidemiology, Microbiology and Immunology. 1992, Vol. 36, No. 2, pages 169-174.	1-35
X,P	ALAEDINI et al. Ganglioside Agglutination Immunoassay for Rapid Detection of Autoantibodies in Immune-Mediated Neuropathy. Journal of Clinical Laboratory Analysis. 2001, Vol. 15, pages 96-99, see entire document.	1-35

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Immunological investigation of chronic inflammatory demyelinating polyradiculoneuropathy

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Abstract

In order to investigate the hypothesis that chronic inflammatory demyelinating polyradiculoneuropathy (CIDP) is an autoimmune disease related to the acute inflammatory form of Guillain-Barré Syndrome (GBS), we studied 40 patients, 40 age and sex matched controls with other forms of peripheral neuropathy (ONP) and 37 controls from the same family or household (FC). We sought antibodies to gangliosides GM1 and LM1 by enzyme linked immunoassay (ELISA) confirmed by immuno-overlay. Only 6 (15%) CIDP patients had IgM antibodies to ganglioside GM1 (GM1) and none had IgG antibodies. We found IgM antibodies to ganglioside LM1 in 2 (5%) and IgG antibodies in 4 (10%) CIDP patients. Antibodies of IgG or IgM class were detected by ELISA to chondroitin sulphate C or sulfatide in up to 7.5% of CIDP patients. There were IgM antibodies in 3 (7.5%) and IgG in 4 (10%) patients against 25, 28 or 36 kD myelin proteins identified by immunoblot. Antibodies to any of these candidate myelin autoantigens were not significantly more frequent in CIDP than FC or ONP controls. Sera from 5 CIDP patients with active disease which subsequently responded to plasma exchange did not induce more demyelination upon intraneural injection into rat sciatic nerve than ONP sera. Serum tumor necrosis factor alpha (TNFα) concentrations were not increased in any of the CIDP patients. Serological evidence of *Campylobacter jejuni* (Cj) infection was present in 4 (10%) CIDP patients. IgM antibodies to cytomegalovirus (CMV) were not detected in any sera. CIDP is not commonly associated with either of these infections or with an antibody-mediated response to any of these glycolipid or myelin autoantigens.

Keywords: Chronic inflammatory demyelinating polyradiculoneuropathy; Antibodies; Myelin proteins; Ganglioside GM1; *Campylobacter*; Cytomegalovirus

1. Introduction

There is a clinical and pathological spectrum ranging from acute, through subacute, to chronic inflammatory demyelinating polyradiculoneuropathy which suggests that they share a common pathogenetic mechanism (Dyck et al., 1993). Injection of myelin in Freund's adjuvant induces autoimmune neuritis in rats which usually has an acute monophasic course with recovery in about 4 weeks but sometimes causes persistent or relapsing deficit providing experimental models of acute inflammatory demyelinating polyradiculoneuropathy (AIDP) and CIDP (Adam et al., 1989). Chronic experimental autoimmune neuritis (EAN) can be induced more readily in rabbits by immunisation with either galactocerebroside (Saida et al., 1981) or large amounts of myelin (Harvey et al., 1987). During the active phases of CIDP, biopsies often show endoneurial inflammatory changes with T-cell infiltration and macrophage-associated demyelination (Schmidt et al., 1996), resembling the changes in EAN. Furthermore activated T-cells and increased concentrations of soluble IL-2 receptor are present in the circulation during relapses (Hartung et al., 1990). These observations and the association of CIDP with other autoimmune diseases (Hughes, 1990), have led to the general hypothesis that CIDP, like AIDP, is caused by an autoimmune reaction to myelin or Schwann cell antigens, possibly triggered by one or several infective agents (Dyck et al., 1993). The search for autoantibodies directed against myelin antigens has been relatively successful in GBS, identifying antibodies to several myelin proteins and glycolipids, especially ganglioside GM1 (GM1) (Hartung et al., 1995a,b), but in CIDP no pathogenetically relevant antibodies have been consistently demonstrated.

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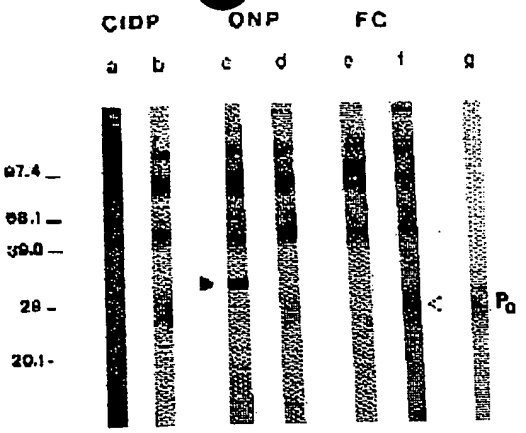

Fig. 1. Detection of IgG antibodies to human myelin proteins by immunoblot following SDS PAGE of human cauda equina extract and transfer onto nitrocellulose. The positions of the molecular weight markers are shown on the left. The last strip (g) was incubated with a mouse monoclonal anti-PO antibody and developed with peroxidase-conjugated anti-mouse IgG antibody. Strips (a) and (b) were incubated with sera from CIDP patients, (c) and (d) from ONP patients and (e) and (f) from family controls subjects. Antibodies to a 36 kD protein (solid triangle) were present in (c) and to a lesser extent in (a). Antibodies to P0 (hollow triangle) were present in (b), (d) and (f). The band at approximately 45–50 kD corresponds to IgG heavy chain and was also seen in control strips incubated with secondary antibody only.

solution were added to the wells of ELISA plates (Linbro, Flow Laboratories) and incubated overnight at 4°C. Wells were blocked with 1% milk powder for 1 h at 37°C and then incubated with 50 μl serum diluted between 1/200 and 1/2000. After washing, antibody binding was detected with 50 μl of 1/2000 anti-human IgG, IgA, IgM biotin conjugate (Chemicon) followed by 50 μl of 1/1000 extravidin–alkaline phosphatase conjugate (Sigma). Sera yielding an optical density (OD) reading at both dilutions higher than the 95th percentile of the household controls of GBS patients in a previous study (Rees et al., 1995b) were tested for individual IgG, IgA and IgM antibodies. Sera were considered positive when they contained high levels of two or more classes of immunoglobulin at both dilutions as previously described (Rees et al., 1995b).

2.2.4. Antibodies to cytomegalovirus

IgM antibodies to CMV were determined by ELISA (Enzygnost Kit, Behring Diagnostics) with CMV infected human fibroblasts and peroxidase-labelled anti human IgM, following precipitation of human IgG anti-CMV antibodies with sheep antibodies directed against the human IgG Fc fragment.

2.2.5. Complement fixation test against human cauda equina

Sera were heat inactivated at 56°C for 30 min and tested for complement fixing activity to a pre-determined optimal dilution of human cauda equina suspension (Hughes et al., 1984).

2.2.6. Tumour necrosis factor α

The concentration of TNFα was measured with an immunoassay (Quantikine High Sensitivity, R&D Systems, Minneapolis). The test serum was added to the well of an ELISA plate which had been pre-incubated with monoclonal antibody to recombinant human TNFα and the amount bound was detected with alkaline phosphatase-linked polyclonal antibody to TNFα in a 'sandwich' assay. The assay incorporated standards in the range 0–32 pg/ml.

2.2.7. Nerve injections

2.2.7.1. Intraneural injection. Intraneural injections were made in the left sciatic nerve of anaesthetised rats using a method previously described (Redford et al., 1995). Groups of 5 male Lewis rats (200–250 g) were injected with each serum under study. We injected sera from 5 CIDP patients who were known to have subsequently improved following plasma exchange by at least one grade on the modified Rankin scale (Rankin, 1957) immediately following collection of that blood sample. Control sera were from patients with other neuropathy which had been stored frozen at −70°C for similar periods. Fresh serum from one additional patient was also injected into 5 animals and compared with serum from a healthy control subject. Seven days after injection, the rats were re-anaesthetised and perfused via the descending aorta with 3% glutaraldehyde (in 0.1 M phosphate buffer). The injected sciatic nerve was removed and processed into resin (Redford et al., 1995) for examination in transverse sections stained with thionine acetate and acridine orange. The number of demyelinated, remyelinated and degenerated fibres at the injection site from 3–5 animals with each serum was counted with an

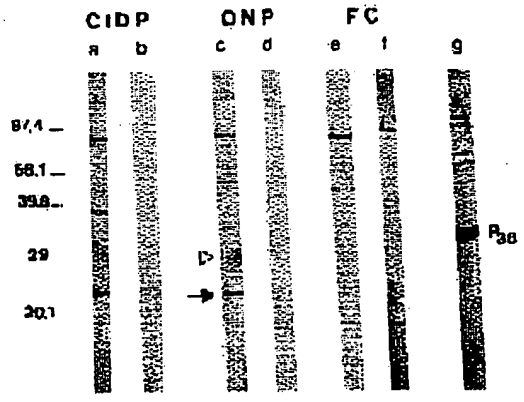

Fig. 2. Detection of IgM antibodies to human myelin proteins by immunoblot following SDS PAGE of human cauda equina extract and transfer onto nitrocellulose. The last strip (g) was incubated with a serum known to contain antibodies to the 36 kD protein. Strips (a) and (b) were incubated with sera from CIDP patients, (c) and (d) from ONP patients and (e) and (f) from family controls subjects. Antibodies to a broad band (hollow triangle) corresponding to P0 and to a 25 kD protein (arrow) were present in (a) and (c). Antibodies to a 25 kD protein were also present in (b), (d) and (e).

eyepiece graticule at a magnification of ×100 by a blinded observer.

2.2.7.2. Electrophysiology. Rats were anaesthetised with 1.5–2% halothane in oxygen and electromyographic (EMG) recordings made from the left sciatic nerve. Recordings were made in all animals immediately before injection of serum and at the end of the study period (7 days) and in 1 animal from each group at 15 min intervals from 30–120 min after the injection of serum. Pairs of stimulating needle electrodes were positioned either side of the sciatic or tibial nerves at the sciatic notch and ankle, respectively, and compound muscle action potentials (CMAPs) evoked by supramaximal stimulation (pulse width 0.1 ms, 1 Hz) were averaged from the dorsal foot muscles. The magnitude and latency of the CMAP following stimulation at either the sciatic notch or ankle were compared. Rectal temperature and the subcutaneous temperature of the left leg were monitored and maintained at 37.0 ± 0.5°C.

3. Results

3.1. Clinical features

We identified 40 patients fulfilling our criteria for CIDP and 40 ONP controls, but only 37 FC since no family or household control was available for 3 patients. The demographic features of the patients are listed in Table 1. There were 27 (14 female and 13 male) patients with a relapsing–remitting course and 13 (11 male and 2 female) with a monophasic or progressive course. The patients with a relapsing and remitting course had an younger mean age at onset (35.1 ± 17.5 years) than those with a chronic progressive or monophasic course (53.8 ± 13.6 years). Two patients, one with a relapsing course and one with a monophasic progressive course, had a pure motor disorder and multifocal conduction block fulfilling not only the neurophysiological criteria for CIDP, but also accepted diagnostic criteria for multifocal motor neuropathy with conduction block (MMNCB) (Bouche et al., 1996). The patients with a monophasic illness had a progressive phase lasting between 10 weeks and 60 months, followed by improvement, with or without treatment, and have had no subsequent relapse.

Table 2
Number (percentage) of patients with CIDP, ONP controls and FC having antibodies to glycoconjugates

Glycoconjugates	Ig class	CIDP (40)	ONP (40)	FC (37)
Ganglioside GM1	IgG	0 (0)	0 (0)	0 (0)
Ganglioside GM1	IgM	6 (15.0)	2 (5.0)	1 (2.7)
Ganglioside LM1	IgG	4 (10.0)	1 (5.0)	0 (0)
Ganglioside LM1	IgM	2 (5.0)	4 (10.0)	1 (2.7)
Chondroitin sulfate	IgG	3 (7.5)	2 (5.0)	0 (0)
Chondroitin sulfate	IgM	1 (2.5)	0 (0)	0 (0)
Sulfatide	IgG	1 (2.5)	0 (0)	0 (0)
Sulfatide	IgM	0 (0)	0 (0)	0 (0)
Any	IgG	5 (12.5)	2 (5)	1 (2.7)
Any	IgM	6 (15)	5 (12.5)	1 (2.7)
Any	IgG/IgM	2 (5)	1 (2.5)	0 (0)

3.2. Antecedent events

Antecedent events were recalled within 4 weeks by 10 patients and consisted of upper respiratory infection in 4 and influenza and gastroenteritis in 3. No patients recalled operations, immunisations or insect bites.

3.3. Antiglycolipid antibodies

Antibodies to glycolipids were only discovered in the serum of a small percentage of CIDP patients and were not significantly more common than in ONP or FC controls (Table 2). The commonest antibodies were IgM antibodies to ganglioside GM1 which were present in 6 CIDP patients, including one of the two with MMNCB, 3 with relapsing and 2 with progressive CIDP. Five of these six patients had disease for 7 to 15 years and one, with the

Table 1
Clinical features of patients with CIDP, ONP controls and FC at the time the serum was tested

	CIDP (40)	ONP (40)	FC (37)
Mean (SD) age (years)	47.0 (18.1)	49.5 (18.6)	46.0 (16.6)
M/F	24/16	24/16	18/19
Infection preceding onset of CIDP	10	—	—
Immunisation preceding onset of CIDP	0	—	—
Mean (SD) age of onset (years)	41.1 (18.4)	—	—
Mean (SD) duration (years)	5.9 (5.0)	—	—
Mean (SD) disability grade	2.7 (1.3)	—	—
On prednisolone	18 (45.0)	—	—
On azathioprine	5 (12.5)	—	—
On IVIg	6 (15.0)	—	—
On PE	4 (10.0)	—	—
On no treatment	15 (37.5)	—	—

weakest GM1 binding, only for 3 months. Antibodies to LM1, chondroitin sulfate and sulfatide were only present in 10% or less of the CIDP patient sera and were not significantly more common than in the ONP or FC subjects.

3.4. Antibodies to myelin proteins

IgG antibodies to a 36 kD myelin protein were detected on Western immunoblots in 2 CIDP, 1 FC and 1 ONP sera. IgM antibodies to this protein were not detected. The highest antibody titre to the 36 kD protein was found in the ONP patient (1/12800) compared to 1/6400 in the CIDP and 1/100 in the FC. Both IgG and IgM antibodies to P0 were found. IgG antibodies to P0 were present in 3 patients with CIDP (2 with relapsing CIDP and 1 with progressive disease), 2 ONP and 3 FC. IgM antibodies to P0 were present in 2 CIDP patients with relapsing disease, 2 with ONP and none of the FC. Additionally, IgM antibodies against an unidentified 25 kD protein were detected in 3 CIDP patients with relapsing disease, 2 ONP and 1 FC. IgM antibodies to this protein occurred in association with IgM antibodies to P0 in 4 out of 6 cases.

3.5. Complement-fixing antibodies to human nerve

Complement-antibodies to human cauda equina were only detected in 1 CIDP serum (titre 1/160) and not in any other sera.

3.6. Serum TNFα concentrations

The mean ($\pm$SD) serum TNFα concentration was 4.2 $\pm$ 2.5 pg/ml in the CIDP patients, which was similar to that in the ONP (6.7 $\pm$ 7.8 pg/ml) and FC (5.0 $\pm$ 6.9 pg/ml) groups. There were no patients in the CIDP group with values greater than the range in the ONP and FC groups.

3.7. Intraneural injections

No change in maximum nerve conduction velocity was observed following intraneural injection of any of the sera tested. Furthermore, the intraneural injection of frozen sera from CIDP or ONP patients had no significant effect on the CMAP amplitude ratio either in the period between 30–120 min after injection, or at 7 days. The mean $\pm$ SD proximal/distal CMAP amplitude ratio for frozen CIDP sera at day 0 was 0.78 $\pm$ 0.08 and 0.75 $\pm$ 0.12 at day 7, while this ratio for frozen ONP sera was 0.78 $\pm$ 0.07 at day 0 and 0.73 $\pm$ 0.09 at day 7. Similar electrophysiological results were obtained after intraneural injection of fresh CIDP serum.

Histological examination revealed that Wallerian degeneration was the predominant pathological feature in all the nerves injected with fresh or frozen CIDP serum, frozen ONP serum or fresh normal serum. The mean number of degenerated or degenerating axons in a single transverse section after injection of fresh CIDP serum was 261 $\pm$ 132, after frozen CIDP serum 197 $\pm$ 172 and after ONP serum 232 $\pm$ 173. Only occasional demyelinated axons (after CIDP serum: 15 $\pm$ 13, ONP: 22 $\pm$ 18) and very few thinly remyelinated axons (CIDP: 7 $\pm$ 7, ONP: 8 $\pm$ 7) were present at 7 days.

3.8. Antibodies to Campylobacter and cytomegalovirus

High levels of two or more immunoglobulin classes against *Campylobacter jejuni* fulfilling our criteria for serological evidence of recent *Campylobacter* infection (Rees et al., 1995b) were detected in 4 CIDP, one FC and none of the ONP sera. IgM antibodies to CMV were not discovered in any of the CIDP patients or controls.

4. Discussion

Despite the generally accepted hypothesis that CIDP is an autoimmune disease and that antibodies or at least serum factors are responsible for its pathogenesis, our studies did not reveal antibodies to candidate myelin antigens in more than a small percentage of patients. We included the full range of clinical courses described in CIDP, including relapsing–remitting and progressive forms, treated and untreated disease, active and remitting stages, mild and severe disability. None of these variables identified a subgroup of patients or stage of the disease in which antibodies to myelin antigens were present in a high proportion. The presence of antibodies was also not related to age or gender.

Only 25% of patients recalled an infective illness during the 4 weeks preceding the onset of their neuropathic symptoms. This agrees with reports of a relatively low percentage of patients in the largest previous series (32/92 (35%)) (McCombe et al., 1987). Symptoms of infection are much less commonly recalled than before GBS, which has been preceded by infections in about two thirds of cases in all series reported (Winer et al., 1988). The low frequency of infections preceding CIDP may not be explained by recall bias in a chronic condition, since records contemporary to the onset were usually studied and the role of infection in the pathogenesis of CIDP must be questioned.

In keeping with the conclusion that infections are not important in the pathogenesis of CIDP is our failure to discover antibodies to *Campylobacter jejuni* significantly more often in the CIDP patients than in the control subjects, or of IgM antibodies to cytomegalovirus in any of the patients. Only limited previous information is available for comparison with these data. Van der Meché and van Doorn (1995) mention an unpublished study in which antibodies to *Campylobacter jejuni* were detected by

counter-immunoelectrophoresis in 47% of 36 CIDP patients and 14% of normal control subjects, but it is unclear whether antibodies detected by this technique detect recent infection. Winer et al. (1988) report a positive IgG immunoassay for cytomegalovirus in 48% of 39 CIDP, 50% of 22 GBS and 8% of 25 controls. Although the proportion with a positive assay was significantly higher than in the normal controls in that series, the absence of IgM antibodies in our own series argues against reactivation of cytomegalovirus infection being important in most cases of CIDP, although relapse of CIDP has been reported following CMV infection (Donaghy et al., 1989). Although these results go some way towards excluding chronic infection or repeated reinfection by these agents as common causes of CIDP, excluding such infection as the initiating event would require a prospective study with sampling of sera soon after the onset.

Antibodies to glycolipids and glycoconjugates were only found infrequently in the sera of our CIDP patients and were not significantly more common than in the control subjects. In particular, antibodies to GM1 were only present in 15% of patients and were of the IgM subclass, by contrast with the presence of antibodies to GM1 in 25% of our series of GBS patients in whom the antibodies were predominantly of the IgG1 subclass (Rees et al., 1995a). This switch to IgG1 indicates T-cell involvement by some unknown mechanism in GBS which does not occur in CIDP, providing a further difference between these conditions. Antibodies to GM1 of the IgM subclass were present in one of the two patients in this series with multifocal motor neuropathy and conduction block and such antibodies have been reported in 20–80% of such patients in previous series (Nobile-Orazio, 1996; Bouche et al., 1996). The limited previous information on the presence of antibodies to glycoconjugates in CIDP (Table 3) is largely consistent with the results of our study, in that antibodies have usually only been reported in a small proportion of patients. We were particularly interested that antibodies to ganglioside LM1 were infrequent, since this is the most abundant ganglioside in human peripheral nerve myelin. Antibodies to ganglioside LM1 were found in a higher proportion of patients in a study which employed a more sensitive TLC overlay assay, but this assay also detected antibodies in normal sera (Fredman et al., 1991). However the overall conclusion has to be that neither of these gangliosides, nor sulfatide, nor chondroitin sulfate are likely to be important auto-antigens in CIDP. In multifocal motor neuropathy with conduction block the possible importance of antibodies to ganglioside GM1 has been supported by reports that sera containing this antibody block conduction in the rat sciatic nerve following intraneural injection (Uncini et al., 1993; Santoro et al., 1992), but this result was not confirmed in a study employing the immunoglobulin fraction instead of whole serum (Harvey et al., 1995). In a sensitive assay with an in vitro mouse phrenic nerve-diaphragm model, sera from patients with multifocal motor neuropathy with conduction block induce conduction block, whether or not they contained detectable antibodies to GM1. This result argues that there are other, as yet unidentified, antibodies which block conduction in multifocal motor neuropathy. The same conclusion can be applied to other forms of CIDP.

Antibodies to peripheral nerve protein were found only in a small proportion of the CIDP patients included in this study (Table 4). Overall only 6/40 (15%) CIDP patients had autoantibodies to P0 and/or to the 36 and 25 kD proteins. The identity of the 25 kD protein is not known. We are currently characterising the 36 kD protein which appears to share its NH_2 terminus with P0, but is not heavily glycosylated and is not recognised by a monoclonal antibody to P0 (Melendez and Gregson, unpublished information). Antibodies to a protein of similar molecular weight were identified in 27/70 (39%) of patients with motor neuron disease (Nobile-Orazio et al., 1994). The clinical significance of the auto-antibodies to these three myelin proteins is dubious as they were also detected in 5/40 (12.5%) ONP patients and in 3/37 (8%) FC subjects. These low percentages of sera having anti-myelin protein antibodies are consistent with previous reports. Antibodies to P0 were detected by ELISA in 5/32 (16%) CIDP, 7/38 (19%) GBS and 2/31 (6%) ONP patients (Khalili-Shirazi et al., 1993). The search for antibodies to P2 and myelin basic protein (Hughes et al., 1984; Zweiman et al., 1983; Khalili-Shirazi et al., 1993) has given positive results in only a small proportion of CIDP patients. We also detected antibodies against multiple high molecular weight bands in most of the patient and control sera. The molecular weights of some of these bands correspond to neurofilament proteins. Auto-antibodies against these components have been found in normal human sera (Stefansson et al., 1985) and were not further analysed in this study. Antibodies to human beta-tubulin, a cytoskeletal protein, were reported to have been detected by ELISA, in 57% of CIDP patients and in only 2% of disease and healthy controls (Hughes et al., 1984). However these results have not been confirmed by two more recent studies (van Schaik et al., 1995; Manfredini et al., 1995) which only confirmed the presence of high titres of anti-beta-tubulin antibodies in 7–10% of CIDP patients when their presence was checked with the more specific immunoblot method. At the 1/100 serum dilution used in our study, we identified a band at 58 kD, which may correspond to beta-tubulin, in most patient and control sera. Others have reported antibodies to beta-tubulin in 86% of sera from healthy control subjects when the serum was tested at 1/200 dilution.

Antibodies to a neuroblastoma cell line, which also cross-react with human peripheral nerve, have been detected by indirect immunofluorescence in 44–58% of patients with CIDP, but only in $< 10\%$ of ONP and healthy controls (Lundkvist et al., 1989; van Doorn et al., 1987). After treatment with intravenous immunoglobulin these

Table 3
Antibodies to glycolipids in CIDP

Antigen	Reference	CIDP no	GBS no	ONP no	OND no	AID no	Normal no	Test
Galactocerebroside	Hughes et al., 1984	1/11 (9%)	2/17(12%)	0/20 (0%)	1/15 (7%)		0/19 (0%)	ELISA
	Rostami et al., 1987	0/11(0%)	0/17(0%)		0/18 (0%)		0/6(0%)	RIA
	McCombe et al., 1988	0/38 (0%)	0/18 (0%)	0/25 (0%)				ELISA
Ganglioside GM1	Baba et al., 1989	3/19 (16%) All 3 had MMNCB			0/19 (0%)		0/10 (0%)	TLC
	Ilyas et al., 1992	2/16 (12.5%)	10/53 (19%)		0/13 (0%)	1/33 (3%)	0/32 (0%)	ELISA and TLC
	Simone et al., 1993	IgG 1/10 (10%) IgM 0/10 (0%)	IgG 9/23 (39%) IgM 4/24 (17%)					ELISA and TLC
	van Schaik et al., 1994	IgG 10/43 (23%) IgA 6/43 (14%) IgM 3/43 (7%)		3/30 (10%) 5/30 (17%) 2/30 (7%)				ELISA
	van Schaik et al., 1995	12/43 (25%)						ELISA
Asialo-GM1	McCombe et al., 1992	IgG 1/4 (25%) IgM 1/1	3/4 (75%) 3/4 (75%)	0/6 (0%) 1/3 (33%)	1/10 (10%) 1/10 (10%)		54	ELISA
Ganglioside LM1	Fredman et al., 1991	10/15 (67%)	10/23 (43%)				8/40 (20%)	TLC
	Ilyas et al., 1992	2/16 (12.5%)	11/53 (21%)		1/13 (8%)	2/33 (6%)	0/32 (0%)	ELISA and TLC
Sulfatide	Fredman et al., 1991	13/15 (87%)	15/23 (65%)				6/40 (15%)	TLC
	Ilyas et al., 1991	1/15 (7%)	11/53 (21%)		Either 5/77 (6.5%)			ELISA and TLC
	Pestronk et al., 1991	0/21 (0%)		18/64 (28%)	0/20 (0%)		0/35 (0%)	ELISA and TLC
	Ilyas et al., 1992	0/16 (0%)	5/53 (9%)		1/13 (8%)	4/33 (12%)	1/32 (3%)	ELISA and TLC
SGPG	Ilyas et al., 1991	1/15 (7%)	13/53 (24.5%)		Either 7/77 (9%)			ELISA and TLC
	Ilyas et al., 1992	0/16 (0%)	7/53 (13%)		0/13 (0%)	2/33 (6%)	0/32 (0%)	ELISA and TLC

Table 4
Antibodies to peripheral nerve and myelin proteins in CIDP

Antigen	Reference	CIDP no	GBS no	ONP no	OND no	AID no	Normal no	Test
Human sciatic nerve	Osuntokun et al., 1966	0/15 (0%)	0/16 (0%)	0/6 (0%)			0/34 (0%)	CFT
	Hughes et al., 1984	0/11 (0%)	2/17 (12%)	1/20 (5%)	0/15 (0%)		0/19 (0%)	CFT
	Toyka et al., 1982	1/1 (case report)		0/1	0/1		0/1	indirect immunofluorescence
	van Doorn et al., 1987	11/22 (50%)	11/16 (69%)	1/34 (3%)	3/32 (9%)	5/39 (13%)	0/25 (0%)	mixed haemagglutination
Peripheral nerve myelin	Latov et al., 1981	2/9 (22%)	3/11 (27%)	0/25 (0%)	0/31 (0%)	0/10 (0%)	0/8 (0%)	CFT (also found in 4/4 para-protreinaemic neuropathy cases)
	Hughes et al., 1984	0/11 (0%)	5/17 (29%)	0/20 (0%)	0/15 (0%)		0/19 (0%)	CFT (rabbit myelin)
	McCombe et al., 1988	1/57 (2%)	12/68 (18%)	0/49 (0%)				indirect immunofluorescence
Myelin basic protein	Server et al., 1979	1/1 (case report)						no demyelinating effect after intraneural injection
Bovine P2 myelin protein	Zweiman et al., 1983	0/13 (0%)	1/18 (5.5%)	0/16 (0%)			0/12 (0%)	ELISA
	Hughes et al., 1984	1/11 (9%)	1/17(6%)	0/20 (0%)	0/15 (0%)		0/19 (0%)	RIA
	Khalili-Shirazi et al., 1993	IgG 4/32 (12.5%)	7/38 (18%)	1/31 (3%)			1/26 (8%)	ELISA
		IgM 12/32 (34%)	15/38 (39.5%)	5/31 (16%)			4/26 (15%)	
Human P0 myelin protein	Khalili-Shirazi et al., 1993	IgG 0/32 (0%)	4/38 (10.5%)	1/31 (3%)			0/26 (0%)	ELISA
		IgM 5/32 (16%)	7/38 (18.5%)	2/31 (6%)			1/26 (4%)	
Schwann cells	McCombe et al., 1988	1/3 (33%)		4/9 (44%)				indirect immunofluorescence
Neuroblastoma	van Doorn et al., 1988	18/42 (45%)	20/48 (42%)	3/43 (7%)	3/46 (6.5%)	1/39 (3%)	0/40 (0%)	indirect immunofluorescence
	Lundkvist et al., 1989	IgG 10/57 (17.5%)	10/71 (14%)	1/68 (1.5%)	3/56 (5%)	1/41 (2%)		indirect immunofluorescence
		IgM 22/57 (39%)	22/71 (31%)	5/68 (7%)	3/56 (5%)	0/41 (0%)		
		Either 30/57 (53%)	31/71 (44%)	5/68 (7%)	4/56 (7%)	1/41 (2%)	0/45 (0%)	
	van Schaik et al., 1995	25/43 (58%)						indirect immunofluorescence
Beta-tubulin	Connolly et al., 1993	IgM 30/70 (42%)						ELISA bovine beta tubulin
		IgG 16/70 (23%)						
		Either 40/70 (57%)	Either 7/35 (20%)		Either 9/483 (2%)			
	Manfredini et al., 1995	IgM 2/20 (10%)	1/56 (2%)	0/46 (0%)	1/79 (1%)	0/38 (0%)	0/26 (0%)	immunoblot human beta tubulin
	van Schaik et al., 1995	3/43 (7%)						immunoblot human beta tubulin

antibodies disappeared from the serum (van Doorn et al., 1988) and $F(ab')_2$ fragments prepared from the immunoglobulin preparation used for treatment inhibited the binding to the neuroblastoma cell line in a dose dependent way (Lundkvist et al., 1989). However the identity of the antigen(s) recognised by these antibodies remains unknown.

Our findings indicate that sera from patients with CIDP do not contain factors which can cause either conduction block or demyelination after their intraneural injection into the rat sciatic nerve. This agrees with the limited previous experiments with CIDP serum. McCombe et al. (1988) were only able to produce demyelination in a similar model with 1 of 11 CIDP sera whereas the same group reported that they were able to produce demyelination with GBS serum (Harrison et al., 1984). In fact, the predominant morphological feature of the nerves injected with either CIDP or ONP sera was axonal degeneration. The degeneration was not simply due to the injection itself, as similar injections of saline into the sciatic nerve of Lewis rats did not produce noticeable histological changes (Redford et al., 1995). It is therefore possible that there are factors, as yet unidentified, present within the sera which are responsible for the degeneration. As most of our observations were made using frozen sera and since it has been reported that freezing can inactivate factors (e.g. complement) within patient sera (Harrison et al., 1984), we also examined a sample of fresh CIDP serum. However, the results obtained using this fresh CIDP serum were indistinguishable from those obtained with frozen CIDP sera, indicating that, in this patient at least, even fresh serum is without demyelinating activity.

The failure of this and previous studies to identify antibodies to myelin antigens which are specific for CIDP is surprising given the strong circumstantial evidence that an auto-antibody mediated mechanism is involved. This evidence includes the efficacy of plasma exchange in treatment (Dyck et al., 1986, 1994), the histological resemblance to galactocerebroside-EAN in rabbits in which complement-fixing antibodies are believed to be important (Saida et al., 1981), elevated levels of soluble terminal complement complex (C5b-9) in the serum in CIDP (Koski et al., 1987) and a report of deposition of complement components on myelin (Dalakas and Engel, 1980). The antibodies which have been identified by us and others in CIDP have also been identified in normal subjects and ONP controls and may not therefore be important factors in inducing disease. It is still possible that these antibodies are involved in pathogenesis and that an additional abnormality in CIDP, either a leaky blood-nerve barrier or a T-cell response, or both, permits abnormal access of serum proteins to nerve fibres. However it is more likely that the relevant antigens have not yet been identified and that unidentified pathogenetically important antibodies do exist. Theoretically cytokines might contribute to demyelination in CIDP since injection of TNFα into rat sciatic nerve induces demyelination (Redford et al., 1995). However there is no evidence to support a role for TNFα since its serum concentration was not increased in our patients, in contrast to the markedly raised concentrations found in the serum of patients with acute GBS (Sharief et al., 1993; Exley et al., 1994). This observation does not rule out a role for local endoneurial cytokine release. It is likely that cellular responses are also involved since there are increased percentages of activated circulating T-cells and increased serum concentrations of soluble IL-2 receptors (Hartung et al., 1990; Taylor and Hughes, 1989). T-cell responses to myelin proteins might repay further study since there is a single report of lymphocyte stimulation by P2 and P0 protein or peptides in 6/13 CIDP patients (Khalili-Shirazi et al., 1992).

The clinical picture in CIDP is difficult to define and heterogeneous in course (relapsing or progressive), distribution of lesions, relative proportions of sensory and motor involvement and response to treatment with steroids, plasma exchange or intravenous immunoglobulin. This heterogeneity is likely to be explained at least in part by differences in underlying pathogenesis and consequently it may be unreasonable to expect a single antigen to be responsible for all cases of CIDP. Perhaps therefore the search should proceed for a number of different antigens with autoantibodies or cell-mediated responses which would explain just a subset of what we presently include under the broader rubric of 'CIDP'.

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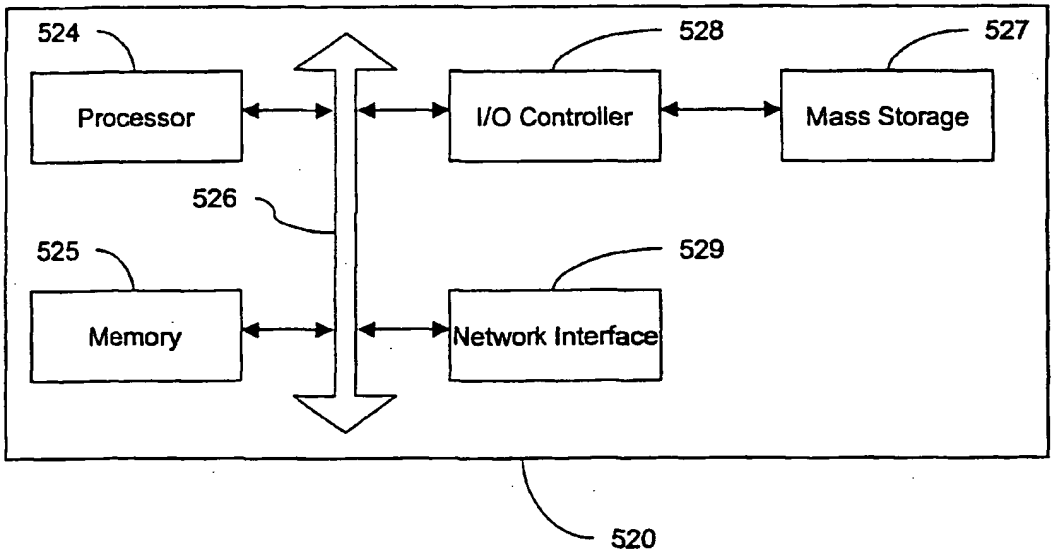
(54) Title: USE OF BLOOD AND PLASMA DONOR SAMPLES AND DATA IN THE DRUG DISCOVERY PROCESS



(57) Abstract: Systems consistent with the present invention provide a method for identifying and recruiting donors whose demographic characteristics, genomic and proteomic profile, and medical histories make them attractive candidates for clinical trials, drug target identification, and pharmacogenomic studies.

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USE OF BLOOD AND PLASMA DONOR SAMPLES AND DATA IN THE DRUG DISCOVERY PROCESS

[001] The present invention relates to methods and systems for identifying individuals for clinical trials. More specifically, the present application relates to a method through which the biopharmaceutical industry can gain access to a large and varied population of individuals with a detailed and fully consented medical history as subjects for the clinical trials required for drug development and as sources of research materials. In another aspect, the present invention relates to a method for creating a longitudinal database of biochemical, genomic, and proteomic information as a resource for drug research and development.

[002] Background of the Invention

[003] Clinical and basic research in the biopharmaceutical industry have the objective of discovery, development, governmental approval, and commercialization of therapies and compounds for diagnosing and treating specific diseases. The phases of discovery, development, approval, and marketing are governed by rigorous laboratory, business, and regulatory standards. The efficient recruitment of patients into studies, however, is often referred to as the Achilles Heel of clinical research.

[004] The multi-billion dollar biopharmaceutical industry continues to struggle to attract the interest of both healthy and diseased individuals to participate in clinical and basic research. Entire companies have been organized to recruit volunteers for studies and to collect biological samples to satisfy research needs. Nevertheless, finding the right individuals either with the targeted disease state or free of the particular disease under study and speeding the process of getting new therapies and medications to market remain serious endeavors. The mechanisms through which study subjects are recruited remain fragmented and uncoordinated.

[005] Clinical trials, which are used to assess the safety and efficacy of potential new diagnostics and therapies, now involve thousands of patients, take years to complete, and cost a great deal. The biopharmaceutical

industry spends hundreds of millions of dollars on patient recruitment for its clinical studies. It is a highly regulated, complex, and traditional industry that goes to extreme lengths to find individuals whose medical profiles fit the needs of specific clinical trials. The biopharmaceutical industry prides itself on its success, yet is always seeking new and productive channels of patient recruitment for its research.

[006] A variety of organizations have varying levels of access to samples or medical data from larger populations. These organizations, however, fail to meet the needs of the biopharmaceutical industry.

[007] Clinical Research Organizations (CROs) have access to patient populations with highly detailed medical records and longitudinal data (participants in Phase I trials often repeat). However, these patients lack ethnic diversity and are targeted to very narrowly defined and limited diseases not usually suitable for discovery purposes. To better characterize issues such as unforeseen toxicity events and non-responders, genomics-based investigation will require samples from larger and more diverse populations than those represented solely in current clinical trials.

[008] Diagnostic companies also have wide population access and some of them have growing genotyping capability. However, they have no long-term sample storage infrastructure. Additionally, these companies do not provide medical characterization, medical histories, or interaction with donors. Because of the lack of this interface, diagnostic companies are unable to sample the donors repeatedly or track their disease progression.

[009] Health Maintenance Organizations' (HMOs) primary shortfalls are that current records are claims-based, rather than medical records, and there are no samples associated with these records and no informed consent for the use of these data in research. While claims and pharmaceutical prescription data provide a privileged perspective of each patient, the medical information needed to monitor patient behavior, such as drug compliance or disease progression, resides with the physician, not the insurance provider. HMOs do not maintain a direct patient interface. Additionally, the perception that HMOs could possibly abuse genotyped samples to discriminate against

patients creates an environment that is not conducive to the collection of family histories, medical records and longitudinal samples.

[010] Life and disability insurers have single-time point medical data and do not store biological samples. Repeat access to medical data typically occurs only when an individual requests an increase in insurance coverage or makes a claim. Therefore, repeat access over time (*i.e.*, longitudinal access) and access to samples are missing from the insurance companies' capabilities. As is the case with HMOs, consent also is an issue for insurers since genetic disease proclivities might be used to discriminate against patients or alter their insurance rates. The claims data processed by insurance companies for statistical purposes do not include personal identifiers or names which could be used to solicit samples.

[011] Sample collectors and specialty blood banks, such as cord blood banks, have access to high quality samples suitable for genetic analysis. However, the samples are frequently collected outside of the context of diseases and are not connected to extensive medical records other than children's birth records. These are often one time samples with no repeat access or possibility for longitudinal analysis and may not have been collected with full disclosure or consent. Most of such specialty blood banks are local and do not draw from a large population base.

[012] Existing genetic population profiling companies, e.g., deCode genetics and Myriad Genetics, target well-defined, but usually inbred, populations in an effort to discover or validate genetic markers linked to disease. Additionally, the target populations tend to be restricted. For example, deCode genetics has access to the medical and genealogical records of the Icelandic population, albeit with only implied informed consent from the individual subjects. Similarly, Myriad Genetics has access to the genealogical records of Mormons in Utah. Neither company has significant access to subjects outside of the target population to verify that candidate genetic markers are relevant to the general population. An example of the misleading conclusions that can result from the use of these selected population datasets is the initial expectation, based on analysis of selected

populations that the BRCA1 mutation was involved in approximately 40% of breast cancers, whereas it is now known that BRCA1 plays a role in only 3%. Furthermore, diseases not prevalent at a high enough frequency in these restricted populations are not addressable.

[013] In contrast, collection establishments enjoy the goodwill and participation of nearly 100,000 individuals each business day. It is well known that blood and plasma donors seek the satisfaction of certain altruistic characteristics through the act of donating. In fact, the safety of a nation's blood supply is typically grounded in the goodwill and honesty of volunteers offering themselves as donors, responding truthfully to medical history questions about their health and certain risk factors in behavior, and the laboratory screening practices for viruses and other diseases known to be transmitted through a transfusion. On average, approximately 15% of those who approach a collection establishment to donate blood are deferred, either temporarily or permanently.

[014] The history of cooperation between the pharmaceutical industry and the blood and plasma industry is well documented, far-reaching, and comprehensive. Without a standing relationship between these industries, blood and plasma organizations would not be able to collect, test, document, and ship products; biopharmaceutical companies would lack significant sales. Professional industry seminars would not be held, nor would numerous physicians, scientists, technologists, and other professionals have access to the latest technology and science in blood and plasma collection and testing. Despite this history of cooperation, however, neither party has developed a method through which the pharmaceutical industry can utilize the sample and data collecting capabilities of the blood and plasma collecting industry to satisfy basic and clinical research needs.

[015] Summary of the Invention

[016] Systems and methods consistent with the present invention provide a new function for the process of donor management in regulated blood and plasma organizations, referred to herein as "collection establishments." To date, the sole purpose of the collection of ancillary blood

samples and personal medical information from blood and plasma donors has been to determine the safety of the procedure for both the donor and the eventual recipient. Most individuals who approach a collection establishment are accepted as donors. Some, however, do not meet the standards for acceptance and are deferred from donating, either on a temporary or on a permanent basis.

[017] Using databases and personal donor relationships conventionally directed toward donor and product safety, the instant invention provides a method through which the substantial data and sample collecting capabilities of collection establishments can be used to identify and recruit subjects for participation in clinical trials. Because collection establishments maintain contact with individual donors over an extended period of time, often years or longer, the invention provides methods through which these same capabilities can be used to identify genomic and proteomic factors that are correlated with the development of disease and/or the response of an individual to drug treatment.

[018] The processes contemplated are (1) the referral of select blood and plasma donors into clinical research studies; (2) the recruitment of blood and plasma donors into clinical research studies; (3) the collection of additional samples and data from donors for use in medical research; and (4) the development of a database comprising the bioinformatic analysis of donor medical histories and biological samples, which can be used to identify genomic, proteomic, and pharmacogenomic correlates of disease and therapeutic response.

[019] BRIEF DESCRIPTION OF THE DRAWINGS

[020] The accompanying drawings, which are incorporated in and constitute a part of this specification, illustrate implementations of the invention and, together with the description, serve to explain the advantages and principles of the invention. In the drawings, dashed lines represent optional elements.

[021] Figure 1 shows a flowchart of steps involved in processing donors from various sources to generate a clinical trial subject database, a

proteomics/genomics/pharmacogenomics database, and a database of biological samples in a manner consistent with the principles of the present invention;

[022] Figure 2 shows a flowchart for processing an end-user generated query to identify clinical trial subjects in the clinical trial subject database in a manner consistent with the principles of the present invention;

[023] Figure 3 is a diagram used to explain how repeated samples from individuals are preserved and tested, either prospectively or retrospectively, for genomic abnormalities and proteomic abnormalities. The disease status of the individuals also is monitored;

[024] Figure 4 shows a system in which methods and systems consistent with the present invention may be implemented; and

[025] Figure 5 shows the components of a desktop or a server computer of the system of Figure 4.

[026] DETAILED DESCRIPTION

[027] Systems and methods consistent withthe present invention provides methods that enable the biopharmaceutical industry to access a large and varied group of individuals whose medical data, for example, demographic characteristics, genetic markers, biochemical markers, family histories, and medical histories, make them attractive candidates for medical research to advance disease diagnostics and therapies. Such systems and methods use a network of non-profit and/or for-profit organizations and partners that have not traditionally been involved in this area of significant medical research as a source for such individuals. For example, a network of collection establishments refers deferred donors and, optionally, accepted donors, into specific clinical studies and collects blood samples and information from both deferred and accepted donors for pharmacogenomic, genomic, or proteomic studies under Institutional Review Board (IRB)-approved procedures and informed consents.

[028] Using systems and methods consistent with the present invention, entities conducting clinical studies have new access to an infrastructure of blood samples, personal medical information and individuals

free of specific diseases and those who may have a specific disease(s) under research. Because individuals often donate blood on a regular basis over long periods of time, *i.e.*, years, the methods of the invention permit the health of donors to monitored over an extended period of time and, furthermore, permit samples to be collected as an individual's medical condition changes.

[029] The ability to propose participation in clinical research to blood and plasma donors enables the biopharmaceutical industry to locate individuals whose disease state, medical histories, and patterns of compliance within a regulated industry result in greater speed through the regulatory approval process and the arrival in the marketplace of life-enhancing diagnostics and therapies for the nation.

[030] The pharmacogenomic interests of the biopharmaceutical industry can also benefit from using systems and methods consistent with the invention. For example, blood and plasma donors' blood and corresponding medical data are used in creating specific genomic and/or proteomic profiles that become benchmarks in the development of diagnostics or therapies for specific diseases. The company looking for the best candidates for a clinical trial on that disease, then focuses enrollment on patients whose profile fits the benchmark. Traditional large Phase III studies are made more efficient. This reduces the time and effort necessary to recruit large numbers of study patients and reduces the cost of drug development for many medicines.

[031] In one implementation consistent with the present invention the problem of recruiting subjects into clinical trials is addressed by providing biopharmaceutical companies with access to a large, diverse population of individuals with well-documented medical histories and detailed clinical profiles. Clinical trial subjects may be recruited from a variety of sources, including, but not limited to, deferred donors and individuals with specific diseases identified through partnerships with physicians and medical centers.

[032] Another implementation consistent with the present invention provides biopharmaceutical companies and researchers with access to a store of biological samples, including, but not limited to, whole blood, serum,

proteins isolated from blood and nucleic acids isolated from blood, obtained with informed consent from a large, diverse population of individuals with well-documented medical histories and detailed clinical profiles. Currently available methods for collecting biological samples from diseased and healthy individuals for genomic and proteomic studies do not reflect the general population because the samples are often from inbred populations with a small founder population. Furthermore, many of these samples are obtained without proper, active informed consent, which is becoming more and more of a concern as the general public becomes aware of the potential monetary value of genetic studies. At present, most readily accessible sample collections represent rather small numbers of individuals and lack the ability to follow-up with the donors through a carefully controlled system that ensures privacy of the donor.

[033] Yet another implementation consistent with the present invention facilitates the study of the inheritance of traits in the context of the entire DNA sequence complement of the organism, a branch of science known as genomics. In addition to analyzing the role of individual genes, genomics seeks to evaluate the importance of potentially highly complex interactions of multiple genes in health and disease. Of further interest is the investigation of an individual's response to treatment with a drug so as to correlate an individual's genetic makeup with drug effectiveness (or pharmacogenomics).

[034] It is believed that, on average, any two individuals differ by only 0.1% in the approximately 3 billion base pairs that make up the genome. This, however, represents as many as 3 million differences, or polymorphisms. In most instances, these polymorphisms represent single base differences, and are thus known as single nucleotide polymorphisms (SNPs). Most of these 3 million or so SNPs lie outside of genes, which comprise only about 3% of the genome, and, in most instances, have no effect on the individual. Even for SNPs that lie within genes, most have no effect on the protein encoded by the gene because of the degeneracy of the genetic code. Benign or silent SNPs, however, may be useful if they co-

segregate with a disease phenotype or if they indicate a specific response to drug therapy.

[035] In some cases, the study of linkage or association of certain genetic markers with the disease state in well-characterized populations has enabled identification of a single gene defect that is both necessary and sufficient for manifestation of the disease. It also has been proven invaluable to have DNA samples from individuals with such so-called monogeneic disease, together with samples from genetically related individuals who do not show signs of the disease. Success also has been seen with populations of well-characterized, unrelated, individuals and matched controls.

[036] The majority of common diseases, however, are rather more complex and are believed to result from the contribution of variations in a number of genes. The combination of certain mutations or polymorphisms can lead to a predisposition to develop a disease, though it is clear that environmental factors also contribute in many instances. In order to understand the etiology of these complex diseases, it is believed that the best approach is to collect large epidemiological study samples from many different populations (Peltonen *et al.*, Science 291: 1224-1229, 2001). One implementation consistent with the present invention facilitates the collection of such large numbers of samples across a varied population.

[037] The study of the human genome has further shown that there may be as few as 30,000 genes in the genome and, therefore, that much diversity must be provided through differences in the synthesis of messenger RNA and, subsequently, protein in different tissues. Consequently, it is important to be able to study the differences in the protein complement of individuals (the proteome) or changes in the posttranslational modification of proteins (both encompassed by the term "proteomics"), particularly any differences between healthy and diseased individuals. Unfortunately, while collections of samples from diseased individuals exist (though often without appropriate informed consent), there are generally no matching samples from those individuals prior to the development of the disease state, which severely limits the types of analysis that can be performed.

[038] Another implementation consistent with the present invention facilitates the identification of such differences in protein expression between healthy and diseased individuals by providing samples from matched groups of healthy and sick individuals. By enabling the provision of large numbers of samples, techniques based on the pooling of samples from one or more groups of individuals can become particularly powerful. Still another implementation consistent with the present invention allows the proteomes of single individuals to be compared before and after disease development. And in a further embodiment, changes in the posttranslational modification of proteins can be investigated in healthy and diseased states.

[039] Yet another implementation consistent with the present invention comprises a longitudinal database in which medical and demographic information for each donor, whether obtained through a collection establishment or through partnerships, is linked to genomic data for that donor, obtained, for example, through SNP analysis, and proteomic data for that donor, obtained, for example, through the analysis of the donor's proteome. These data are correlated with the subject's disease status and stored in a proteomics/genomics database. The samples collected from an individual over time for example, from that individual's first sample donation through either the development of disease in or death of that individual, also are stored and may be retrieved by accessing a longitudinal database of samples. The database may be queried in order to identify genomic and/or proteomic changes associated with the development of disease. Furthermore, as the database comprises vast amounts of data from large numbers of individuals, researchers are able to query the database in a hypothesis-free manner, as well as with hypothesis-driven queries. For instance, the vast amount of data can be queried for unexpected correlations of certain genomic and proteomic characteristics with disease phenotypes.

[040] Another implementation consistent with the invention facilitates drug target identification and validation. Traditionally, potential drug targets have been identified on the basis of hypotheses from biochemical or pharmacological study of the disease state. Genomics allows the expansion

of this approach to include searching the genome for genes encoding proteins with particular characteristics, or motifs, suggestive of classes of receptors or other classical drug targets or the study of changes in the expression of different nucleic acids. Alternatively, analysis of DNA samples for patterns of SNPs can be used to determine whether certain genotypes are associated with a particular disease, which may in turn lead to the identification of a new drug target. This latter approach requires samples of DNA from subjects with the target disease, together with a matched set of "healthy" controls.

[041] Yet another implementation consistent with the invention facilitates research into the individual variability in response to drug treatment, which is a consequence of the genomic make-up of the individual. The study of this variability in response to drugs and its relation to the genetic markers (SNPs) in an individual provide the opportunity for selection of the most appropriate treatment, in terms of both efficacy and safety. This approach, known as pharmacogenomics, plays an increasingly important role, not only in the selection of the most appropriate treatment for an individual, but also in drug development by enabling the selection of the most appropriate subjects for clinical trials.

[042] Reference will now be made in detail to implementations consistent with the present invention as illustrated in the accompanying drawings. Wherever possible, the same reference numbers will be used throughout the drawings and the following description to refer to the same or like parts.

[043] Definitions

[044] The term "collection establishment" as used herein refers to any blood or plasma organization contemplated as part of the invention. Collection establishments are typically regulated by the Food and Drug Administration or a similar agency. A collection establishment can be either an independent entity or owned by the contractor.

[045] The term "end-user" as used herein means any entity that requests the names of donors or deferred donors fitting the profile for clinical trial subjects. End-users also include any entity that orders blood and/or DNA

samples from a collection establishment for pharmacogenomic purposes and any entity that uses the longitudinal database of genomic/proteomic information.

[046] The term "contractor" as used herein refers to an entity that acts by contract as an intermediary between collection establishments and end-users. A contractor may be an end-user. The contractor queries collection establishments for individuals or samples that meet the criteria established by an end-user and arranges the supply of contact information or of those samples to the end-user. The contractor also provides end-users with access to databases according to the invention. The contractor may audit end-users to ensure the proper use of the information or samples by the end-user under the terms of the contract. The contractor's role as an intermediary does not preclude the contractor from undertaking additional functions of the invention including, but not limited to, sample preparation, storage, and shipping, SNP analysis, and proteomics analysis.

[047] The term "donor" as used herein means an individual who offers to donate or sell blood, plasma, or serum to a collection establishment. Donors fitting particular profiles also may be identified through partnerships with physicians, medical centers, and other health care providers.

[048] The term "deferred donor" as used herein means an individual who offers to donate or sell blood, plasma, or serum to a collection establishment, but whose offer is refused, either temporarily or permanently, based on medical history or other relevant information.

[049] The term "longitudinal" as used herein means obtained over a period of time. When the term "longitudinal" is applied to an individual or group of individuals, the period of time, in general, extends from an individual's first to last sample donations. The last sample donation may occur, for example, when the individual develops a disease, when the individual begins treatment of a disease, or upon the death of the individual. When the term "longitudinal" is applied to a sample or to information, the period of time may extend beyond the death of the individual from whom the sample or information was gathered.

[050] As used herein, the term "pharmacogenomics" pertains to the correlation between an individual's response to treatment with a drug and that individual's genetic makeup. The term may be encompassed within the more general term "genomics".

[051] Overview of System Components and Operation

[052] The implementation consistent with the invention may comprise a contractor, a network of collection establishments and, optionally, partners, and end-users. As exemplified below, systems consistent with the invention may be implemented using a computer network. Those skilled in the art will appreciate, however, that a manual implementation also may be consistent with the present invention. Systems consistent with the present invention enable end-users, for example, biopharmaceutical industry consumers, to select clinical trial participants, DNA samples, and tissue samples from subjects suitable for drug development studies and clinical trials. Suitable subjects will vary from study to study and may be selected based on criteria such as age, sex, ethnicity, or race. The skilled artisan will recognize, of course, that many other selection criteria also may be appropriately applied depending on the particular requirements of the study.

[053] Donor Information And Sample Collection

[054] As diagrammed in Figure 1, multiple collection establishments 101, 105, and 110 are intake sites for prospective donors 125, optionally in collaboration with one or more partners 115 and 120. The collection establishments obtain informed consent 127 from prospective donors in compliance with Institutional Review Board-approved procedures permitting, for example, the use of donated tissue samples in biomedical research and/or the release of the information needed to contact an individual to pharmaceutical companies seeking clinical trial subjects or research subjects. The collection establishments also collect donor demographic information, family histories, and medical histories 140 and 145, and, optionally, perform clinical chemistry analyses on donor samples 150 (any and all such information being generally defined as "medical data"). Table 1 provides examples of the type of information requested from prospective donors and

the types of clinical tests performed on the blood of prospective donors. A non-exclusive list of other possible tests, which may be performed either singly or in various combinations, are included in an Appendix .

Table 1

Demographic Information
- donor name
- donor social security number
- donor address and zip code
- donor phone – work and home
- donor birth date
- donor race
- donor gender
- donor employer

Donation Profile
- date of last donation
- total number of donations
- blood (ABO/RH) type

Health History
- weight
- temperature
- pulse
- blood pressure
- hemoglobin/hematocrit
- recent flu
- recent cold
- recent sore throat
- skin problems
- rashes
- any immunization
- chest pain
- heart disease
- lung disease
- cancer
- blood disease
- bleeding problem
- yellow jaundice
- hepatitis
- malaria
- Chagas disease
- babesiosis
- under a doctor's care
- recent surgery
- recent dental work
- taking any medication
- taken human growth hormone
- taken Tegison
- taken Accutane
- taken Proscar
- syphilis
- gonorrhea
- pregnant
- blood transfusion
- organ transplant
- tissue transplant
- tattoo
- ear or skin piercing
- contact with another's blood
- exposure to hepatitis
- exposure to Creuzfeldt-Jacob disease
- used a needle to take drugs
- given money for sex
- given drugs for sex
- taken money for sex
- taken drugs for sex
- sex with someone who has taken money for sex
- sex with someone who has taken drugs for sex
- men – sex with a man since 1977
- women – sex with a male who had sex with a man since 1977
- taken clotting factor concentrate
- sex with someone who has taken clotting factor concentrate
- AIDS
- positive test for AIDS
- sex with someone who has AIDS
- sex with someone who has HIV antibody
- travel outside U.S. or Canada
- born or lived in African countries since 1977
- received blood transfusion in African country
- had sex with someone from African countries
- transfusion-associated AIDS
- transfusion-associated Hepatitis

Laboratory Screening Tests

- antibody screening results
- alanine aminotransferase (ALT)
- Cytomegalovirus (CMV) screening
- Hepatitis B screening
- Hepatitis B Core Antibody screening
- Hepatitis C screening
- Human immunodeficiency virus (HIV) Types 1 & 2 screening
- Human T-cell lymphotropic virus (HTLV)-1 screening
- HIV Antigen screening (9)

Additional data maintained on plasma donors

- breastfeeding now
- close contact with someone with jaundice
- Varicella-Zoster (live)
- Hemophilus Influenza type B-
- PCR test (HAV, HBV, HCV, HIV, Parvovirus B 19)
- Serum protein electrophoresis (SPE)
- tetanus
- prison or jail in past 12 months
- atypical Anti-D Antibody
- antibiotics within the past 14 days
- urinalysis

[055] Additional information of use to the end-user may be collected, either prospectively or retrospectively. One skilled in the art will readily recognize that the nature of the donor information requested is dictated by the requirements of the study in which the donated sample is to be used.

[056] The information collected is gathered by any available mechanism, including, but not limited to, confidential, personal interviews, the use of self-executed forms, or even by direct entry into a computerized database, for instance via a personal computer terminal or via a hand-held device. The information collected from prospective donors may be generally the same as is collected at present by collection establishments and is maintained in confidence.

[057] The existing infrastructure of the blood and plasma industry may be employed to collect information from donors. Individuals collecting information are trained to comply with Standard Operating Procedures (SOPs) developed for the business. The training of individuals responsible for collecting donor information is documented and entered into the individual's permanent personnel record. Individuals collecting information from donors are located either at the site of the collection establishment or at one or more remote locations separate from the point of contact for blood and plasma donors. These individuals also are equipped to explain and administer informed consents. The informed consent describes, for example, the fact that information of a personal and/or familial nature is requested by an end-user, for example, a pharmacogenomic, biotechnology, or pharmaceutical company, developing treatment or drugs to help cure specific diseases. If the nature of the disease to be studied is known, this information may be disclosed in order to engage the interest of the donor.

[058] Informed consents are maintained by the collection establishments, or, alternatively, by a contractor, preferably in donor files. It is not contemplated that the names or informed consents of individual donors are disclosed to clients. Rather the collection establishment provides the client with evidence of informed consent, for example, a Verification of a

Signed Informed Consent Form accompanying donor-derived samples. If desired, audits performed at the request of the client and, preferably, conducted by an independent third party, assure the client that proper informed consents have been administered. In addition, since most of the data collected from donors is entered into a computer, all appropriate firewalls of confidentiality and privacy are, of course, employed. The signature for the Informed Consent may be implemented using digital signature techniques.

[059] To further protect the identity of donors, the invention employs alphanumeric strings, rather than names, to identify each donor. Such strings may be assigned by either the contractor, the collection establishment, or the client. The collection establishment may assign unique, confidential identification numbers to donors. The collection establishment may also assign a unique, confidential identification number to each sample collected from a donor. Presently, a unique one-time number is assigned to the product donated in both the whole blood and the plasma industries. In implementation consistent with the invention, these numbers are used to identify sample and donor information.

[060] Based on the prospective donor's answers and test results, the individual is classified either as an accepted donor 130 or as a deferred donor 135. Medical history and clinical testing data along with the results of proteomic and genomic analyses of both accepted and deferred donors are combined to make the proteomics and genomics database 155.

[061] The clinical trials database 160 comprises data collected from deferred donors. Optionally, the clinical trials database also may comprise data collected from accepted donors

[062] Data collected from donors are kept in perpetuity. As requested by an end-user and in compliance with an IRB-approved informed consent, donors are, from time to time, asked to supply additional and/or updated information. All such updates are incorporated into the permanent record of the donor.

[063] Method For Identifying Clinical Trial Subjects

[064] One implementation of the invention provides a method for identifying a research subject, comprising: a) obtaining medical data from a subject; b) associating an identifier for said subject with said medical data in at least a first database; c) associating the identifier for said subject with the name and contact information of said subject; d) identifying criteria for selecting a research subject; e) extracting an identifier from the first database, wherein said identifier is associated with a subject matching the identified criteria; and f) matching the identifier from the first database with the name and contact information in order to identify the research subject.

[065] A request to identify potential clinical trial subjects originates with an end-user 201 (see Figure 2). The end-user provides desired subject characteristics 210 to the contractor 215. For example, the end-user may wish to identify individuals with specific pharmacogenomic characteristics, e.g., relating to a cytochrome P450. Based on those characteristics, the contractor formulates a query 220, which is designed to interrogate the clinical trials database 160 for subjects with the desired characteristics. The query is sent to Server A, which comprises the clinical trials database, over a communications network 230. Records in that database that satisfy the query are identified 240 and output as unique patient identifiers by Server A 250.

[066] In one implementation consistent with the invention, the name and contact information associated with each identifier also are stored in the clinical trials database 160.

[067] In another implementation consistent with the invention, the name and contact information associated with each identifier are stored in a second database, which cross references the unique patient identifiers with the names and contact information of the corresponding individuals.

[068] In one implementation consistent with the invention, the clinical trials database and the second database are stored on Server A. In another implementation, the second database is stored on a separate Server B 270. In implementations of the invention utilizing Server B, Server A may be either directly linked to Server B through a firewall 260 or, alternatively, freestanding

and without links to other components of the communications network. Information is retrieved from Server B either through the communications network if a link is present in the system or manually if Server B is freestanding.

[069] In general, the contractor or the collection establishment contacts individual identified and seeks permission to pass patient contact information 280 on to the end-user. Alternatively, the patient information 280 may be sent directly to the end-user, who then contacts the individuals identified or, alternately, further refines the query for resubmission to the contractor.

[070] Although the invention does not contemplate directly releasing data, other than names and contact information, supplied by individual donors to end-users, donors are, on occasion, asked for permission to release demographic information. Such demographic information is only released in confidence to end-users and without disclosing the identity of the individual(s) from whom that information was collected. Additionally, from time to time, and with donor consent, the results of donor testing for viruses, including, but not limited to, hepatitis B virus (HBV), hepatitis C virus (HCV), and human immunodeficiency virus (HIV), are disclosed to end-users.

[071] Method For Establishing A Proteomics/Genomics Database

[072] As illustrated in Figure 1, biological samples 150 are collected from both accepted and deferred donors. The sample collected is generally whole blood, but other tissues may be collected, especially in collaboration with partners. Portions of each sample are stored as whole blood or as any fraction of whole blood (e.g., serum, lymphocytes, erythrocytes, etc.) and as nucleic acids derived from such whole blood or fraction of whole blood. Donor DNA and RNA are extracted using methods, either manual or automated, known to those skilled in the art.

[073] Donor samples are stored under standard conditions known in the art, preferably at a centralized depository maintained by the contractor, although storage at multiple sites, which may be maintained by third parties, is consistent with the invention. In one embodiment, stored samples are bar-

coded with unique identifiers to facilitate their identification and retrieval from storage. The facility for sample handling and storage may include a system for robotic handling and retrieval of individual samples.

[074] As illustrated in Figure 3, samples 301, 311, 321, 331, 341, and 351 are collected from the same individuals repeatedly over time, in general over years. These samples are stored as described above and constitute a longitudinal sample database 305. The longitudinal sample database comprises at least 2 samples, and may comprise at least 50, at least 1000, at least 10,000, at least 500,000, at least 1,000,000, at least 5, 000,000, or at least 10,000,000 samples. Samples are retrieved from the longitudinal sample database on demand to satisfy the needs of the contractor or of an end-user.

[075] In addition to the data in Table 1 and, optionally, additional information from other tests, for example, listed in the Appendix,, which are associated with each sample, genomic experiments 312, for example, to detect SNPs or to monitor changes in gene expression, and proteomic experiments 315, for example, to detect aberrant protein expression or changes in the posttranslational modification of proteins, are performed on each sample either at the time the sample is acquired or retrospectively, for example to search for changes in DNA sequence, RNA expression, or protein activity that are associated with a later-arising disease 318.

[076] An example of information that may be stored in the proteomic/genomics database is shown in Figure 3. Assays performed on samples 301 and 311, which are collected from the same individual at different times, show a DNA polymorphism (e.g., a SNP), but show normal RNA and protein expression. At the times samples 301 and 311 are collected, the individual shows no sign of disease. Assays performed on samples 321 and 331, again collected from this individual but at later times, as before show a DNA polymorphism and now also show abnormal expression of at least one protein and/or RNA. The amount of abnormal expression increases between the date sample 321 is collected and the date sample 331 is collected. At the time sample 341 is collected, the individual

has begun to show disease symptoms. The DNA polymorphism persists and the extent of abnormal protein/RNA expression has increased. The DNA polymorphism persists in sample 351, but the abnormal protein and/or RNA is more or less abundant. Disease severity has worsened at the time sample 351 is collected, suggesting that the DNA polymorphism and the expression abnormality may be diagnostic for the disease and may be therapeutic targets.

[077] Databases

[078] Donor information and data associated with samples (e.g., storage location, SNP profile, etc.), collectively "information," may be stored using any method that permits high productivity, scalability, flexibility, accessibility, security, correctness and consistency of housed data, data granularity, and presentation. The storage system may be a computerized database. In one implementation consistent with the invention, the information is stored in a secure, computerized data warehouse system, accessible only by controlled passwords assigned to trained users. In general, collection establishments currently use this type of system for data storage. The data warehouse is designed using dimensional modeling, a logical design technique that seeks to present the data in a standard framework that is intuitive and allows for high-performance access. This type of modeling provides the optimal balance among critical factors such as productivity, scalability, flexibility, accessibility, security, correctness and consistency of housed data, data granularity, and presentation.

[079] A centralized database of information is generally maintained by the contractor, although systems for housing all or part of a database may be distributed at different sites.

[080] In one implementation consistent with the invention, end-users provide the contractor with criteria through which the desired donors and samples may be identified. The contractor causes the donor data and sample information database or databases to be searched using queries developed using the client-supplied criteria. Standard query protocols are used, resulting in the data required for the end-user. In general, a query tool

set is selected that allows for services such as warehouse browsing, query management, standard reporting, access and security.

[081] Database queries are performed by trained employees either of the contractor or of the collection establishments. Database queries may be performed by the contractor, by employees of the collection establishments, who, as part of their normal jobs, query the databases for routine purposes of the collection establishments, or by end-users, following protocols establishing confidentiality and proper security. The result of a query is the approach to an individual donor to participate in a client's research, the shipment of sample to the client, or the identification of desired proteomic/genomic information.

[082] It will be appreciated that the present invention may be implemented in a software system, which is stored as executable instructions on a computer readable medium accessible either directly or through a network. Figure 4 illustrates a conceptual diagram of a computer network 400 in which methods and systems consistent with the present inventionmay be implemented to permit users to query a database of donor and sample information. Computer network 400 comprises one or more small computers (such as desktop computers , 410, 420, and 425) and one or more large computers (such as Server A 412 and server B 422). In general, small computers are "personal computers" or workstations and are the sites at which a human user operates the computer to make requests for data from other computers or servers on the network. Usually, the requested data resides in the large computers, but the size of a computer or the resources associated with it do not preclude the computer's acting as the home of a database. In one implementation consistent with the invention, Servers A and B are connected through a firewall 435, which permits secure access to information that identifies donors to authorized users. In another implementation consistent with the invention, Servers A and B are not connected by a network and patient information must be accessed directly from server B.

[083] Desktop computer systems and server systems compatible with the invention includes conventional components, as shown in Figure 5, such as a processor 524, memory 525 (e.g., RAM), a bus 526 which couples processor 524 and memory 525, a mass storage device 527 (e.g., a magnetic hard disk or an optical storage disk) coupled to processor 524 and memory 525 through an I/O controller 528 and a network interface 529, such as a conventional modem or Ethernet card.

[084] The distance between a server 412 and a desktop computer 410 may be very long, e.g., across continents, or very short, e.g., within the same building. When the distance is short, the network 400 is preferably a local area network (LAN). When the distance between server 412 and desktop computer 425 is long, the network 400 may, in fact, be a network of networks, such as the Internet. In traversing the network, the data may be transferred through several intermediate servers and many routing devices, such as bridges and routers. Proper security and flexibility of access will be employed to provide authorized access through commonly used interface technologies.

[085] The software system of the present invention is, for example, stored as executable instructions on a computer readable medium on the desktop and server systems, such as mass storage device 527, or in memory 525. Access to the system described above is available on a single-use or on a multiple-use basis. Preferably, end-users contract with the contractor for continuing access to the system.

[086] The foregoing description of implementations of the invention has been presented for purposes of illustration and description. It is not exhaustive and does not limit the invention to the precise form disclosed. Modifications and variations are possible in light of the above teachings or may be acquired from practicing of the invention. For example, the described implementation includes software but the present invention may be implemented as a combination of hardware and software or in hardware alone. The invention may be implemented with both object-oriented and non-

object-oriented programming systems. The scope of the invention is defined by the claims and their equivalents.

APPENDIX

1,1,1-Trichloroethane, Blood
1,25-Dihydroxy Vitamin D3
11 DEOXYCORTISOL
11-Desoxycortisol
17-Hydroxycorticosteroids
25-Hydroxycalciferol
3-A-ANDROSTANEDIOL
3-Methoxy-4-Hydroxymandelic Acid
4-AMINOANTIPYRINE
5' Nucleotidase
5-HIAA
5-NUCLEOTIDASE
5T Allele Genotyping
A, Vitamin
A-1 Apolipoprotein
A1A Phenotyping
A1-Antitrypsin
A-1-ANTITRYPSIN
Abnormal Bleeding Profile
ABO
ABSCESS AFB CULTURE
AC Globulin
ACA
ACE
ACH R AB
Acid Anhydride Profile
ACT LOW RANGE-MEDTRONIC
ADENO AG BY DFA
ADH
ADRENAL ANTIBODIES
ADVIL
AEROBIC CULTURE
AFB BLOOD CULTURE
AFP
AGBM
ALA
Albumin
ALCOHOL PANEL
ALDOLASE
Alk Phos
ALPHA 2 ANTIPLASMIN
ALT
ALUMINUM
AMA
AMIKACIN
AMMONIA
AMNIOTIC FLUID
AMOBARBITAL
AMPHETAMINE
AMYLASE

APPENDIX

ANA
ANCA
ANDROSTENEDION
ANGIOTENSIN CONVERTING ENZYME
ANTI GBM
APC Mutations in FAP
APOLIPOPROTEIN A1
APTT
ARGININE VASOPRESSIN
ARSENIC
ASCORBIC ACID
ASMA
ASO (ANTI-STREPTOLYSIN O) TITER
ASPARTATE AMINOTRANSAMINASE
AST
AT III
a-Thalassemia
AURAMINE STAIN
AUTOMATED DIFFERENTIAL
AVP
B CELL CYTOTOXIC CROSSMATCH
B SURFACE ANTIGEN
B VIRAL DNA
B.PERTUSSIS CULTURE
B1 Vitamin, Plasma
B12
B-19 PCR
B27
B2-MICROGLOBULIN
B6, Vitamin
Bacterial Antigens (Serum, Urine, Cerebrospinal Fluid)
BAL FOR CMV CULTURE
BANDS
BARBITAL
Basic Metabolic Panel
BAYER ENCORE QA PLUS, GLUCOSE
BB
B-Cell Gene Rearrangements, Ig Heavy Chain
BCL-2 t(14;18) Translocation
BCR-ABL t(9;22) Translocation
BCSF
BENADRYL
Beta Apolipoprotein
Beutler-Baluda Test
BGP
B-hCG Quantitative, Serum
Bi, Blood
BICARBONATE
Bile Acids
Bioavailable Testosterone

APPENDIX

BISCODYL
Bladder Tumor Antigen (BTA) Cytology Profile
BLEEDING TIME
Blood Acetone
BODY FLUID
Bone Alkaline Phosphatase (BAP)
BORDETELLA PERTUSSIS CULTURE
BRAIN BIOPSY
Breast
BROMPHENIRAMINE
BRUCELLA AGGLUTININ
BSAB
BSF-2
BTA
b-Thalassemia (Cooley's Anemia, Mediterranean Anemia
BUFFY COAT FOR CMV
BUN
BUPROPION
BUTABARBITAL
C, Vitamin
C. DIFF TOXIN
C.DIFF CULTURE
C1 Esterase Inhibitor
C1EI
C1Q
C2 Complement
C3
C3c
C4
C4, Body Fluid
Ca
Ca, Urine
Ca++, Ionized, Serum
CA-125
Cachectin
CADASIL
CAE
CAFFEINE
CALCITONIN
cAMP, Urine
Canavan Disease, DNA Analysis
C-ANCA-Specific Antibody
Cancer Antigen (CA) 125
CARBAMAZEPINE
CATECHOLAMINES
CBAVD
CBC
Cd, Blood
CD25
CD3

APPENDIX

CD4
CD8
CDF
CEA
Celiac Disease (CD) Antibodies
Centrax®
c-erbB-2
CEREBROSPINAL FLUID (CSF) CULTURE
CF Carrier
CH50
Chain-of-Custody Protocol, Specimen
CHEM 10
Chickenpox Culture
CHLAMYDIA
Chol
Christmas Factor
Circulating Anticoagulant
CITRATE
CK
CK, Serum
CK-2
Cl
Cl, CSF
CLB Smear
Clearance, Creatinine
Clomipramine (Anafranil®), Serum
CMV
CO, Blood
CO2
COAG FACTOR CONCENTRATES
Cobalamin, True
COCAETHYLENE-COCAINE ANALOG
Codeine
Coke
Cold Agglutinin Titer, Quantitative
Combined Esterase (CES)
COOMBS CROSSMATCH
COPPER
CORD ISOHEMAGGLUTININS
COTININE
Coumadin®
Coxiella burnetii Antibodies
C-PEPTIDE
CPK
CPPT
Cr, Plasma
Crack
C-REACTIVE PROTEIN
Creat
CRM Assay

APPENDIX

CROSSMATCH TEST EA UNIT
CRP
CRYO
CSA
CSF AFB CULTURE (SpinalFl)
cTnI
Cu, Plasma
CULTURE FOR B.PERTUSSIS
Cutaneous Immunofluorescence, Indirect
CVS Prenatal Karyotyping, Chromosome Analysis
CYANIDE
CYCLIC ADENOSINE MONOPHOSPHATE
Cyst Fluid Amylase
CYTOLOGY, "RUSH" OR "SAME DAY"
D
D Factor
D XYLOSE 5 HOUR TOLERANCE URINE
DALA
DANTHRON
DARKFIELD EXAM
DAT
DAZ+ Analysis
DBili
DCC Allelotyping
D-DIMER
DDT Exposure Profile
DEHYDROEPIANDROSTERONE
DELTA
DEMEROL
DEOXYCORTICOSTERONE
DEPAKENE
Dermatophyte Culture
DES
Dexedrine®
DFA FOR CHLAMYDIA
DHEA
DHT
Diazepam, Serum
DIBUCAINE
DIC Profile
DIFF
Digitalis
Dihydrotestosterone
DIL
Dimethylacetamide Exposure Profile
DIPHENHYDRAMINE
DIRECT ANTIGLOBULIN
Disease Association
DITHIONITE TEST
DMAC

APPENDIX

DMD/BMD
DNA Analysis for Parentage Evaluation
Dolophine®
DOPAMINE
DORIDEN
DOUBLE-STRANDED DNA
DOXEPIN
Dpd
D-PYRALINKS
DQ1
DR Transplant
DR2
DRAINAGE CULTURE
DRVVT
DS-DNA
Duraquin®
d-Xylose Absorption Test
E. coli O157:H7
E.HISTOLYTICA CULTURE
E1
E2
E3, Serum
Ear Culture
EBV
ECG Cardiologist Overread Only, Adult
ECTODERMAL DYSPLASIA (LINKAGE ANALYSIS)
ED STAT PANEL A
Effusions Cytology
EGFR
EHEC, Stool Culture
ELAVIL
Electrolyte Panel
ELISA ANTIBODY SCREEN
ELUTION
ELVIS®
Endep®
ENGRAFTMENT STUDY
ENTAMOEBA HISTOLYTICA CULTURE
Environmental Culture
Eos Count
EP
EPG SERUM
EPHEDRINE
Epidermal Growth Factor Receptor (EGFR)
EPO
Epstein Barr Virus (EBV) Antibodies
ER/PR Assay
Erythrocyte Count
Eskalith®
ESOPHAGEAL BRUSHING CYTOLOGY

APPENDIX

ESR
Essential and Metabolic Fatty Acids Analysis
Esterase Inhibitor
ETHANOL (NOT FORENSIC)
EtOH
Etrafon®
Excedrin®
Extrinsic Factors
Eye
F, Plasma
FA FOR B.PERTUSSIS
Factor B
Familial Adenomatous Polyposis (FAP)
FANA
FAP Analysis, Known Mutation
Farmer's Lung
Fast Hemoglobins
FAT STAIN
FBS
FDP, Plasma
Fe
FECAL CULTURE
FELBAMATE
Female Hormone
FEP
FERN TEST
FETAL FIBRONECTIN
FFP
FIBRIN SPLIT PRODUCTS
FINE NEEDLE ASPIRATE CYTOLOGY
FIORNAL
FISH
FK506
FK-506
FLECAINIDE
FLOW ANTIBODY SCREEN
FLU A
FMR-1 Gene Trinucleotide Repeat Analysis
FOLATE
Fourth Complement Component
FPL
Fractionated Amino Acids, 24-Hour Urine
Free and Albumin-Bound Testosterone
Friedreich Ataxia (FRDA)
FROZEN PLASMA
Fructosamine
FS
FSH
Ft Bragg Fever
FT4

APPENDIX

FTA-Ab
FULL CROSS
Functional Protein C
G-1-PUT
G6PD
G-6-PD QUANTITATIVE
GABAPENTIN
GAD AUTOANTIBODIES
GALACTOSE
GAMMA GLUTAMYL TRANSFERASE
GANGLIOSIDE GM1 ANTIBODIES
Garamycin®
Gastric Analysis
Gaucher Disease, DNA Analysis
GC (Neisseria gonorrhoeae) Culture Only
GENERAL VIRAL CULTURE
Germ Cell Panel
Gestational Diabetes Evaluation
GGT
GHB
GIARDIA FA
GIEMSA STAIN
Glass Activation Factor
Gliadin Antibodies
Globulins, 24-Hour Urine
Glu
GLYCATED ALBUMIN
GM1 ANTIBODIES
GM2 Gangliosidosis
GMS/FUNGAL SILVER STAIN
Gonorrhea Culture
Goodpasture Syndrome
GOT
GPT
GRAM SMEAR, DIRECT
Gross and Microscopic Pathology
GT
GTT
Gynecologic Pap Smear and Maturation Index
H and E Sections
H. FLU GROUP B LATEX AGGLUTINATION
H.PYLORI AB
H2 RECEPTOR ANTAGONIST
H2a/H2b and H3 and H4 Antibodies
HAA
Haemophilus influenzae B Antigen
Hageman Factor
Hairy Cell/Plasma Cell Leukemia Profile
Haldol®
HAMA

APPENDIX

HANE Assay
Haptoglobin
HAV/HBV (Profile VII)
Hb A1c
HBcAb, IgG/IgM Diff
HBeAb
HBSAB
HBV DNA Qualitative PCR
HCG
HCT
HCV Ab (Immunoblot Reflex)
HDL
HDV
Heat Precipitate Fibrinogen
HEINZ BODY STAIN
Helicobacter pylori Antibodies
Hema-Chek®
HEP AM
HER-2/neu Gene Amplification
Heterophil Agglutinins
Hexagonal Phase Phospholipid
Hg, Blood
HGB
HGF
HGH
HHV-6, IgG
High Resolution G-Banding
HIPA
Hirsutism Prof, Comprehensive
HISTAMINE
HITS
HIV
HLA A Typing
HNPCC, Direct, Known Mutation
Hog
HOLD SPECIMEN
Homocyst(e)ine, Plasma or Serum
Hormonal Evaluation Cytology
HPV
HS CRP
HSF
HSV
HTLV
Human Antimouse Antibodies
HUNTINGTON DISEASE MUTATION
HVA
Hybrid Capture
Hyperhomocysteinemia, C677T Mutation
IA2 Antibodies
IA-9

APPENDIX

IAA
IBC
IBUPROFEN
ICA512 Autoantibodies
ICSH
Ictotest®
Identification of Atypical Bacteria
IFE/Protein Electro, 24-Hr Ur
Ig Heavy Chain Gene Rearrangement
IGA
IgD
IgE
IGF BINDING PROTEIN 3
IGG
IGM
IL-2 sR
Imavate®
IMIPRAMINE
IMMEDIATE SPIN CROSSMATCH
In situ Hybridization for HPV
Inborn Errors of Metabolism
Inclusion Body Stain
INDIA INK (SpinalFl)
INFECTIOUS MONO
Inherited Mental Retardation
Inorganic Phosphate, Blood
INR
Insulin
Interleukin-2 Receptor
IONIZED CALCIUM
IRON
IS CROSSMATCH
ISLET CELL ANTIBODY
Isoagglutinins
ITRACONAZOLE LEVEL
IVY BLEEDING TIME
Ixodes Tick Bite Agent
Jembec Culture
Jewish Heritage
JO 1
JOINT FLUID
JUMBO FFP
K
K
K and L Chains, Urine
Kappa Light Chains, Urine
Karyotype
KENNEDY DISEASE DNA

APPENDIX

Ketone Bodies, Serum
Ki67
Kidney Stone
Killer Weed
KLEIHAUER-BETKE
KlonopinTM
KOH
L/S Ratio
Labile Factor
Lactate
Lambda Light Chains, Urine
Lanoxin®
LAP
LASA
Latex
LAXATIVE ABUSE SCREEN
L-Carnitine, Total, Free, and Esters
LCM
LD
LD, Body Fluid
LDH
LDL
LEAD
Lecithin/Sphingomyelin Ratio
Legionella Antibodies, IgM
LEISHMANIA CULTURE
LEPTOSPIRA CULTURE
Lesion Culture
Leu3A
LGV
LH
LHRT
Li, Blood
Librax®
LiCO3
LIDOCAINE
Li-Fraumeni Syndrome (p53)
LILEY CURVE, AMNIOTIC FLUID
LIPASE
Liquiprin®
LITHIUM
Liver Cancer Monitor Profile
LKM-1 Antibodies
Loa loa Smear
Long chain 3-hydroxyacyl-CoA dehydrogenase
LORAZEPAM
Loss of Heterozygosity
Low-Density Lipoprotein Cholesterol (Direct)
LOXAPINE
LP

APPENDIX

Lp(a)
LRP
LSD Screen, Urine
LUKENS-TRAP
Lumbar Puncture
Lung, Adenocarcinoma Monitor Profile
Lupus Anticoagulant
Luteinizing Hormone (LH) and (FSH)
Lyme Disease (Borrelia burgdorferi) by PCR
Lysergic Acid Diethylamide, Urine
M PNUEMONIA ANTIBODY
MAC BLOOD CULTURE
MAGNESIUM
MAI BLOOD CULTURE
MALARIA
Manganese, Blood
MAPROTILINE
Marijuana
Maternal Serum Alpha-Fetoprotein
MAXIMUM BACTERICIDAL DILUTION
MBC
MBD
MBK, Blood
MBP
MCH
MCV
MDA
MDMA
MEAN CELL HEMOGLOBIN CONCENTRATION
Mebaral®
MECONIUM DRUG SCREEN
Medium chain acyl CoA dehydrogenase (MCAD)
Megaloblastic Anemia, Serum
MEK, Blood
Melanoma Monitor Profile
MENINGOCOCCUS GROUPS (A,B,C,Y,W135)
Meperidine (Demerol®), Serum
MERCURY
Mesoridazine (Serentil®), Serum
Metabolic Dysglycemia Profile
Mexate®
Mg, RBC
MGC GROUP B (SpinalFl)
MHA-TP
MIA Test
MIBK, Blood
MIC
Midstream Urine Culture, Routine
MILTOWN
MINIMUM BACTERICIDAL CONCENTRATION

APPENDIX

Miscellaneous Fluid Cytology
Mitochondrial Ab
MMAC
MMR
Mn, Blood
MNBK, Blood
MODIFIED ACID FAST STAIN
Mold Culture
MONO SCREEN
Morphine
MOTRIN
MPO-ANCA
MRSA Culture
msAFP
MTB, PCR (With Culture)
MTD
MTX, Blood
Mucin Clot Test
Multiple Endocrine Neoplasia (MEN2A)
MUMPS
MURAMIDASE TEST
MUSCLE BIOPSY
Myasthenia Gravis Antibody
MYCO -M
Myelin Basic Protein (MBP), Cerebrospinal Fluid
Myidone®
MYOGLOBIN
MYSOLINE
Na
NA
N-ACETYLPROCAINAMIDE
NAPA
NARCOLEPSY ASSOCIATED ANTIGEN
Nasal Smear for Eosinophils
NATIVE DNA
Navane®
NBT
N-DESALKYLFLURAZEPAM
Nebcin®
Necropsy
Neisseria gonorrhoeae by DNA Probe
NEMBUTAL
Neopterin
NERVE BIOPSY
Neuroblastoma Monitor Profile
NEWBORN HEMOLYTIC DISEASE WORKUP
NF-1, Known Mutation
NGI SuperQuantTM
NH3
NH4

APPENDIX

Ni, Plasma
Nickel, Plasma
Niemann-Pick Disease, DNA Analysis
Nipple Discharge
NITROBLUE TETRAZOLIUM, CGD
NKH1A, Leu19 (for CD56)
N-Methylacetamide
NMP 22
Nongonococcal Urethritis Culture
Noradrenaline, Plasma
Nose Culture
NSE
N-Telopeptide
NTX Test
Nuclear Matrix Protein (NMP) 22
O AND P
O2CT
OB HOLD REQUEST
OCCULT BLOOD
Ocular Cytology
OD 450
OGTT
OKT3 (CD3)
Oligoclonal Banding
ON SERVICES/ BLOOD BANK
ONE HOUR GDM
Opiate Confirmation, Urine
ORAL CYTOLOGY
Orbinamon®
Organism Identification
Ornithine transcarbamylase deficiency (linkage assay)
Oropharyngeal Brushings Cytology
Osmol
Ostase®
OVA AND PARASITE
OXALATE URINE
Oxidative Stress Analysis
OXYCODONE
P
P AND P TEST
P. carinii Pneumonia, Stain
p24 Antigen
PABA
Packed Cell Volume
PAIGG, PAIGA, PAIGM
Pamelor®
P-ANCA-Specific Antibody
Pancreatic Cancer Monitor Profile
PAP
PARA 3 AG DFA

APPENDIX

PAS
Paternity Studies
Pb, Blood
PBG DEAMINASE, ERYTHROCYTE
PCB Exposure Profile, Plasma
PCO2
PCP
PCR - CMV (SpinalFl)
PCV
PEDIGREE RECONSTRUCTION
PENTAZOCINE
PEPSINOGEN
PG and Creatinine, Amniotic Fluid
PG, Amniotic Fluid
PH
pH, Body Fluid
Phagocytosis
PHENAZOPYRIDINE
Philadelphia Chromosome
Phos
PHYTANATE
Pi Phenotype
Pill Analysis
Pinworm Preparation
Pituitary Glycoproteins, Alpha Subunit
PKU
PLACENTA EXAMINATION
PLEURAL FLUID
PLP
PLT
PNEUMO LATEX AGGLUTINATION
PO2
PO4
POLIO 1 ANTIBODY
PORCINE FACTOR VIII INHIBITOR TITER
Postmortem Examination
POTASSIUM
PP Glucose, Two-Hour
PPH
PPLO Antibodies
PPP
PR3-ANCA
PRA
Prealbumin
PRIMIDONE
Proaccelerin
PRUSSIAN BLUE STAIN
pS2 Protein, Paraffin Block
PSA
PSEUDOCHOLINESTERASE

APPENDIX

PSITTACOSIS
PT
PTH Intact
PTT
Punch Biopsy
PURKINJE CELL CYTOPLASMIC AB TYPE 1
PUS CULTURE
PYRALINKS-D
Q FEVER
QUAALUDE
Quick-Cult®
RA Latex
Random Blood Glucose
Rapid Grower Susceptibility Testing
Rash Profile A
RBC Cholinesterase
Recombinant Immunoblot Assay
Red Blood Cell (RBC) Antigen
Reference Bacterial Culture Identification
Rela®
Renal Function Panel
Replication Error
RER
Resistance Analysis
RET Mutations In MEN 2 And FMTC
Reverse T3
RF Assay
Rh Factor
Rheumatic Fever Profile
Rh-hr Genotype
Rho(D) Typing
RIBA HCV
Rickettsia rickettsii Titer
Ristocetin Cofactor
Ritalin®
RMSF IgM Antibodies
Rocket Fuel
Rotavirus, Direct Detection by Immunoassay
Routine Culture, Stool
RPR
RSV by DFA
Rubella Antibodies, IgG
S. pneumoniae Antigen
Saccharomonospora viridis
Salicylate, Serum
Sandimmune®
Sb, Urine
Scleroderma Diagnostic Profile
Scotch®
Se, Blood

APPENDIX

Secobarbital
Sedimentation Rate, Westergren
Selenium, Blood
Semen Analysis, Basic
Sensitivity Testing
Serentil®
Seven Drugs Plus Ethanol
SEX DETERMINATION
SGOT
SGPT
SHBG
Shingles Culture
Sickle Cell Anemia (Hb SS or SC)
Siderophilin
Silver, Plasma
Sinequan®
Sjögren's Antibodies (Anti-SS-A/Anti-SS-B)
Skeletal Alkaline Phosphatase (SALP)
Skin Biopsy ((To be assigned by pathologist))
SLE
SM-C/IGF-1
Smooth Muscle Antibodies
Snow
Sodium Fluoride
Soluble Transferrin Receptor
Soma®
Soprodol®
Soridol®
SPCA
Specific Esterase
Spinal and bulbar muscular atrophy (SBMA)
Spontaneous Abortion Chromosome Analysis
Sputum Culture
SRY/AZF Determination
ssay sensitivity)
St Louis Encephalitis Virus Antibodies, IgG
Stable Factor
Sterile Body Fluid Culture
sTfR
STH
Stimulation Test
Stool Culture
STR Analysis
STS
Stuart Factor
Styrene Exposure Profile
Sudan Black B
Sugar, Quantitative, Urine
Sulfate, Quantitative, 24-Hour Urine
SuperQuantTM HCV

APPENDIX

Surface Factor
Susceptibility Testing
Swamp Fever
Swineherd Disease
Synovial Fluid, Mucin Clot Test
Syphilis Serology
Systemic Lupus Erythematosus (SLE) Profile A
T- and B-Cell Gene Rearrangements
T Cell Receptor Beta Chain (TCRß)
T3
T3, Free
T4
T4, Free by Equilibrium Dialysis, Serum
T4/T8 Analysis
TAC Antigen
Tambocor®
Tape Test
Tartrate-Resistant Acid Phosphatase Stain
Tay-Sachs Disease, Biochemical, Leukocytes
TB Stat Test
TBG
TBili
TCA, Urine
T-Cell Activation Profile, CD8 Subsets
TCK
TCO2
TeBG
Tegretol®
Teichoic Acid Antibodies
Tempra®
Testicular Cancer Monitor Profile
Tetanus Antibodies
Thallium, Urine
THC
Theo-Dur®
Thiamine, Plasma
Thorazine®
Throat Culture
Thyrocalcitonin
TIBC
Tissue Karyotype
Titratable Acidity
Tl, Urine
TLI
T-Lymphocyte Helper/Suppressor Profile
TNF
Tobramycin (Nebcin®), Serum, Peak
Tofranil®
Toluene Exposure Profile
Topiramate (Topamax®), Serum

APPENDIX

Total Bili
Toxoplasma gondii Antibodies, IgG
TPO Antibodies
TProt
TRAb
Treponema pallidum Antibodies (FTA-ABS)
Triavil®
t-RNA Synthetase
Troponin I
True Cholinesterase
Trypsin
TSH Receptor Ab
TSI
Tularemia Agglutinins
Tumor Necrosis Factor-
Twin Zygosity, Pre- and Postnatal
Tylenol®
Type and Rh
Tyrosine Phosphatase Autoantibodies
Tzanck Smear
UA
UA, Routine
UIBC
UltraQualTM HCV
Unbound T3
Unconjugated DHEA
Undifferentiated Tumor Panel
Uniparental Disomy Profile
Unsaturated Iron Binding Capacity
Upper Respiratory Culture, Routine
Urea Clearance
Uric A
Uroporphyrin
Uterine Cancer Monitor Profile
UUN Clearance
Vaginal Cytology
Valium®
Vancocin®
Varicella-Zoster Virus (VZV) Antibodies, IgG
Vasoactive Intestinal Polypeptide (VIP), Plasma
VDRL, Cerebrospinal Fluid
VGCC Antibody
VIP
Viral Culture, General
Viscosity, Serum
Vitamin A and Carotene
VMA and Catecholamines
Volatiles, Blood
von Recklinghausen Disease
VRE, Culture Only

APPENDIX

vW Factor Assay
vWF Antigen
VZV by DFA
Warfarin (Coumadin®), Serum
WBC
WBlot HIV1
Weil Disease
Westergren Sedimentation Rate
Wet Prep
White Blood Cell (WBC) Count
Whole Blood Histamine
Wood Alcohol
Wound
Wright-Giemsa Stain
Wuchereria Smear
Xylenes Exposure Profile
Yeast Culture
Zarontin®
Zinc, Plasma/Serum
Zn, Serum
ZPP
ZYGOSITY TESTING

We claim:

1. A method for identifying a research subject, comprising:

- obtaining medical data from a subject;

- associating an identifier for said subject with said medical data in at least a first database;

- associating the identifier for said subject with the name and contact information of said subject;

- identifying criteria for selecting a research subject;

- extracting an identifier from the first database, wherein said identifier is associated with a subject matching the identified criteria; and

- matching the identifier from the first database with the name and contact information in order to identify the research subject.

2. The method according to claim 1, further comprising obtaining informed consent from said subject, wherein said informed consent permits the medical data to be used to identify said subject as a potential research subject.

3. The method according to claim 1, wherein medical data are obtained from said subject and associated with the identifier for said subject in at least a first database longitudinally.

4. The method according to claim 1, wherein said subject is a member of a group of donors, and said method is repeated for each member.

5. The method according to claim 1, wherein the subject is a deferred donor.

6. The method according to claim 1, wherein the medical data comprise a medical history.

7: The method according to claim 1, wherein the medical data comprise a family history.

8. The method according to claim 1, wherein the medical data comprise clinical chemistry test results.

9. The method according to claim 1, wherein the medical data comprise pharmacogenomic or genomic data.

10. The method according to claim 1, wherein the medical data comprise proteomic data.

11 The method according to claim 1, wherein the criteria include medical history information.

12. The method according to claim 1, wherein the criteria include family history information.

13. The method according to claim 1, wherein the criteria include clinical chemistry test results.

14. The method according to claim 1, wherein the criteria include pharmacogenomic or genomic information.

15. The method according to claim 1, wherein the criteria include proteomic information.

16. The method according to claim 1, wherein said first database is a computerized database.

17. The method according to claim 1, wherein the name and contact information is stored in at least a second database.

18. The method according to claim 17, wherein said first database and said second database are computerized databases.

19. The method according to claim 18, wherein the first and second databases are stored on separate computers.

20. The method according to claim 19, wherein the computer storing the first database is connected through a network firewall with the computer storing the second database.

21. The method according to 1, wherein the first database is a computerized database and is accessible through a network.

22. The method according to claim 21, wherein the network is a local area network or an intranet.

23. The method according to claim 21, wherein the network is an internet.

24. A method for identifying a research subject in a group of donors from at least one collection establishment, comprising:

a. obtaining a biological sample and medical data from a donor;

b. associating an identifier for said donor with said biological sample and medical data in at least a first database;

c. associating the identifier for said blood donor with the name and contact information of said donor;

d. identifying criteria for selecting a research subject;

e. extracting an identifier from the first database, wherein said identifier is associated with a donor matching the identified criteria; and

f. matching the identifier from the first database with the name and contact information in order to identify a research subject.

25. The method according to claim 24, further comprising obtaining informed consent from said blood donor, wherein said informed consent

permits the medical data to be used to identify said blood donor as a potential research subject.

26. The method according to claim 24, wherein medical data are obtained from said donor and associated with the identifier for said donor in at least a first database longitudinally.

27. The method according to claim 24, wherein said donor is a deferred donor.

28. The method according to claim 24, wherein the medical data comprise a medical history.

29. The method according to claim 24, wherein the medical data comprise a family history.

30. The method according to claim 24, wherein the medical data comprise clinical chemistry test results.

31. The method according to claim 24, wherein the medical data comprise pharmacogenomic or genomic data.

32. The method according to claim 24, wherein the medical data comprise proteomic data.

33. The method according to claim 24, wherein the criteria include medical history information.

34. The method according to claim 24, wherein the criteria include family history information.

35. The method according to claim 24, wherein the criteria include clinical test results.

36. The method according to claim 24, wherein the criteria include pharmacogenomic or genomic information.

37. The method according to claim 24, wherein the criteria include proteomic information.

38. The method according to claim 24, wherein said first database is a computerized database.

39. The method according to claim 24, wherein the name and contact information of said blood donor is stored in at least a second database.

40. The method according to claim 39, wherein said first database and said second database are computerized databases.

41. The method according to claim 40, wherein said first and second databases are stored on separate computers.

42. The method according to claim 41, wherein the computer storing the first database is connected through a network firewall with the computer storing the second database.

43. The method according to 24, wherein the first database is a computerized database and is accessible through a network.

44. The method according to claim 43, wherein the network is a local area network or an intranet.

45. The method according to claim 43, wherein the network is an internet.

46. A plurality of biological samples collected from at least one subject, wherein each sample is associated with an identifier linking said biological sample to at least one of medical data, genomic data, pharmacogenomic data, and proteomic data in at least a first database and wherein said biological samples are collected and stored longitudinally.

47. The plurality of biological samples according to claim 46, wherein said samples are whole blood, plasma, serum, blood cells, and proteins or nucleic acids isolated therefrom.

48. A plurality of biological samples collected from at least one donor, wherein each sample is collected at a collection establishment and associated with an identifier linking said donor and said biological sample to at least one of medical data, genomic data, pharmacogenomic data, and proteomic data in at least a first database and wherein said plurality of biological samples are collected and stored longitudinally.

49. A method for creating a database, the method comprising:

a. collecting a biological sample from at least one subject;

b. collecting a medical data from said at least one subject;

c. deriving proteomic information and genomic information from the sample;

d. storing the sample in a location from which the sample can be recovered;

e. associating the medical data, the proteomic information, and the genomic information with an identifier that can be used to locate the sample; and

f. performing steps a to e on the same subject longitudinally; and wherein steps b to d may be performed in any order.

50. The method according to claim 49, wherein steps a to f are performed on multiple subjects.

51. The method according to claim 49, wherein the biological sample is whole blood, plasma, serum, blood cells, or proteins or nucleic acids isolated therefrom.

52. The method according to claim 49, wherein the samples are collected from at least one collection establishment.

53. The method according to claim 49, wherein said medical data comprises clinical chemistry test information.

54. The method according to claim 53, wherein the clinical chemistry test is at least one test selected from ABO/RH type, antibody screening tests, alanine aminotransferase (ALT) tests, cytomegalovirus (CMV) screening, hepatitis B screening, hepatitis B core antibody screening, hepatitis C screening, human immunodeficiency virus (HIV) types 1 and 2 screening, human T-cell lymphotropic virus (HTLV)-1 screening, and HIV antigen screening.

55. The method according to claim 49, wherein the genomic information includes DNA polymorphisms.

56. The method according to claim 49, wherein the DNA polymorphisms are single nucleotide polymorphisms.

57. The method according to claim 49, wherein the proteomic information includes the proteins expressed in the sample.

58. The method according to claim 49, wherein the genomic information includes the ribonucleic acids expressed in the sample.

59. The method according to claim 49, wherein said medical data comprises family histories from the subjects.

60. The method according to claim 49, wherein said medical data comprises demographic information from the subjects.

61. The method according to claim 49, wherein at least one of the medical data, the genomic information, the proteomic information, and the location for the sample is associated with an identifier for the subject that can be used to retrieve the name and contact information of said subject

62. A method for identifying a genomic or a proteomic characteristic which correlates with a disease, said method comprising:

- creating a database according to claim 48;
- identifying subjects with the disease;
- identifying genomic and proteomic characteristics shared by said subjects.

63. The method according to claim 62, wherein the genomic characteristic identified is a single nucleotide polymorphism.

64. The method according to claim 62, wherein the genomic characteristic identified is pharmacogenomic information.

65. The method according to claim 62, wherein the proteomic information is a change in protein level.

66. A method for recruiting a research subject for a clinical study, said method comprising:

- identifying said research subject according to claim 1 according to selected criteria; and

- contacting said research subject for recruiting said research subject for said clinical study.

67. A method for recruiting a research subject for a clinical study, said method comprising:

- identifying said research subject according to claim 24 according to selected criteria; and

- contacting said research subject for recruiting said research subject for said clinical study.

68. The method according to claim 27, wherein said deferred donor is a deferred blood or plasma donor.

Figure 1

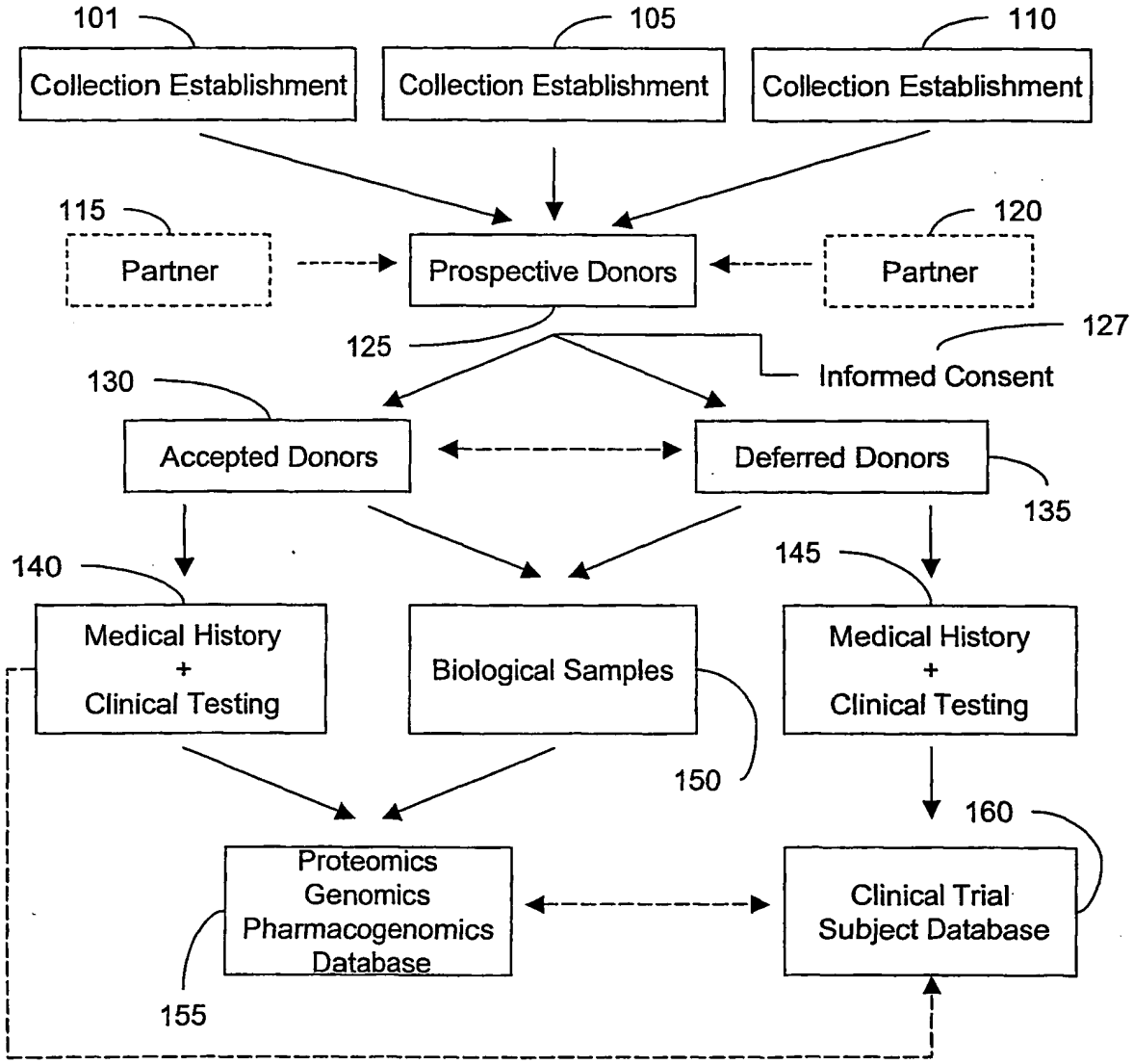

101
Collection Establishment
105
Collection Establishment
110
Collection Establishment
115
Partner
Prospective Donors
120
Partner
125
127
Informed Consent
130
Accepted Donors
Deferred Donors
135
140
Medical History
+
Clinical Testing
Biological Samples
145
Medical History
+
Clinical Testing
150
160
Proteomics
Genomics
Pharmacogenomics
Database
Clinical Trial
Subject Database
155

Figure 2

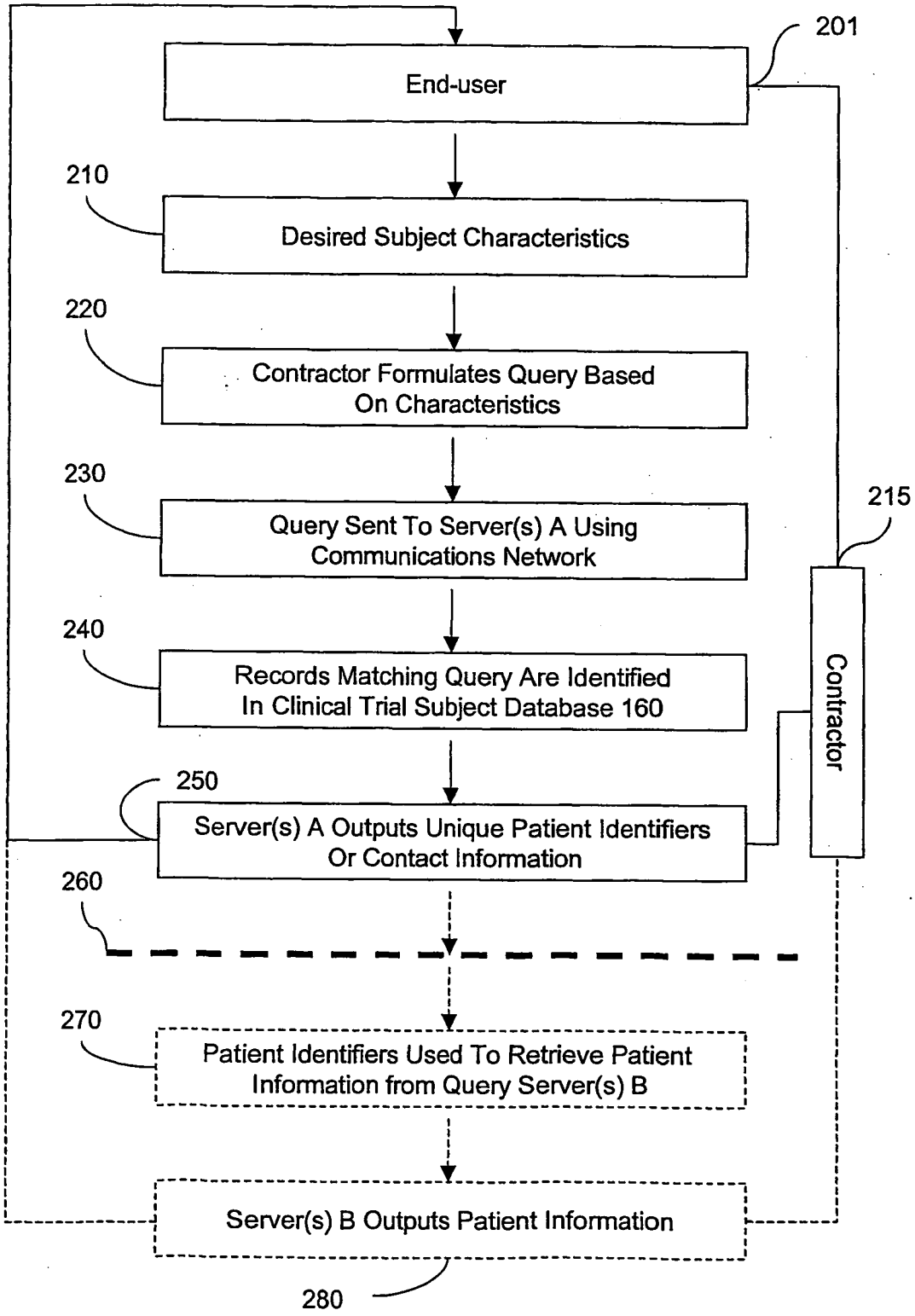

201
End-user
210
Desired Subject Characteristics
220
Contractor Formulates Query Based On Characteristics
230
Query Sent To Server(s) A Using Communications Network
215
240
Records Matching Query Are Identified In Clinical Trial Subject Database 160
Contractor
250
Server(s) A Outputs Unique Patient Identifiers Or Contact Information
260
270
Patient Identifiers Used To Retrieve Patient Information from Query Server(s) B
Server(s) B Outputs Patient Information
280

Figure 3

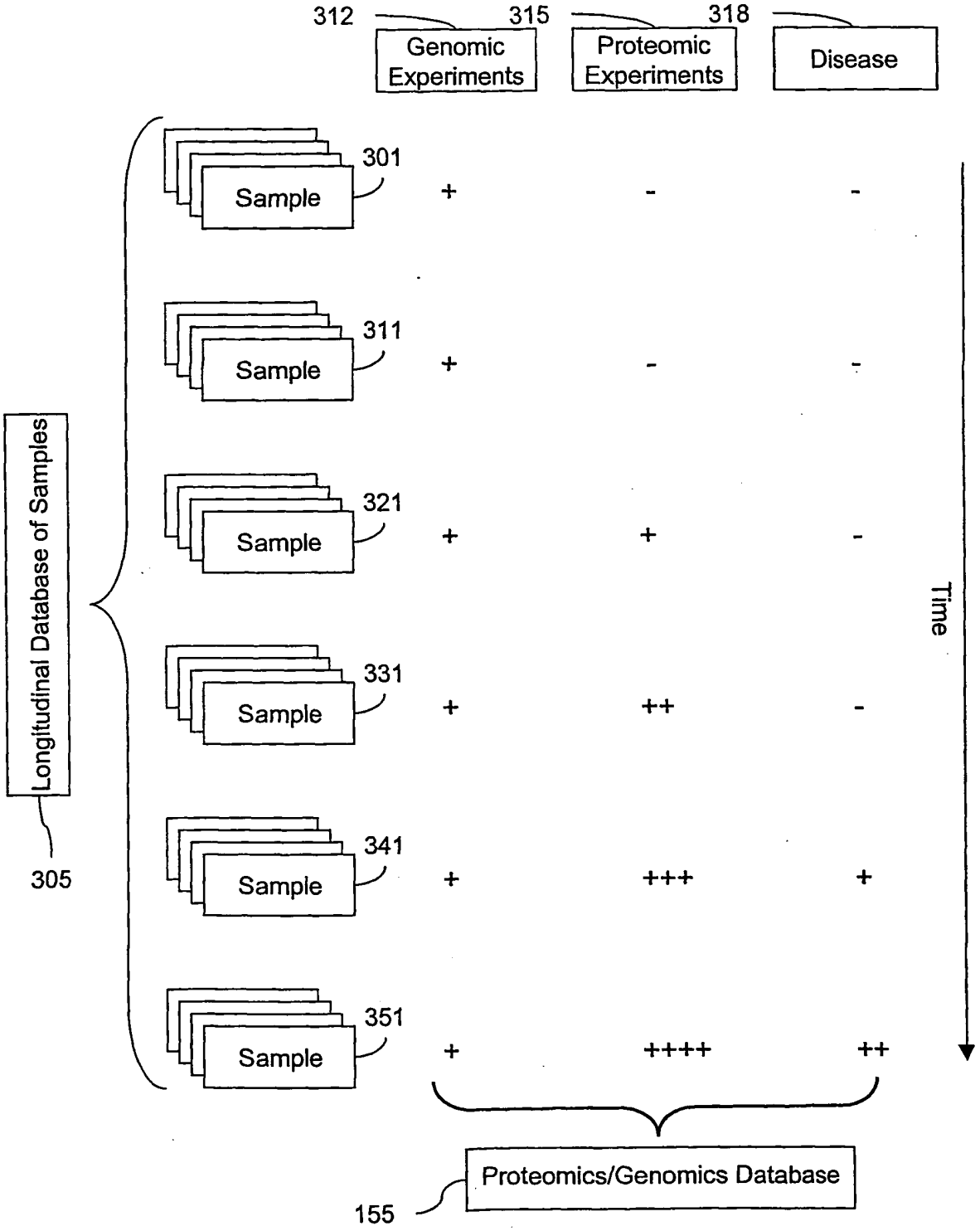

312
315
318
Genomic Experiments
Proteomic Experiments
Disease
301
Sample
+
-
-
311
Sample
+
-
-
321
Sample
+
+
-
331
Sample
+
++
-
341
Sample
+
+++
+
351
Sample
+
++++
++
Longitudinal Database of Samples
305
Time
Proteomics/Genomics Database
155

Figure 4

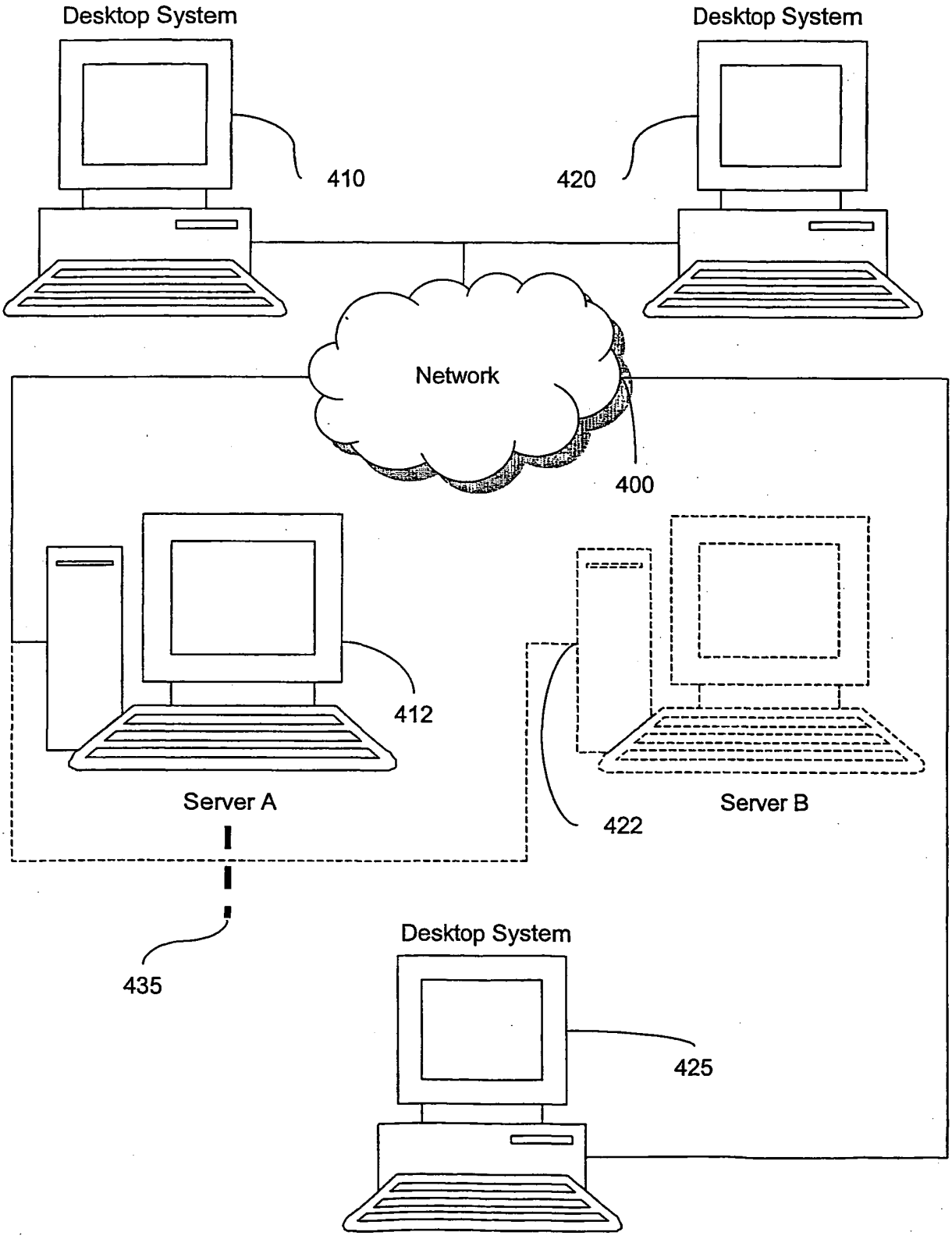
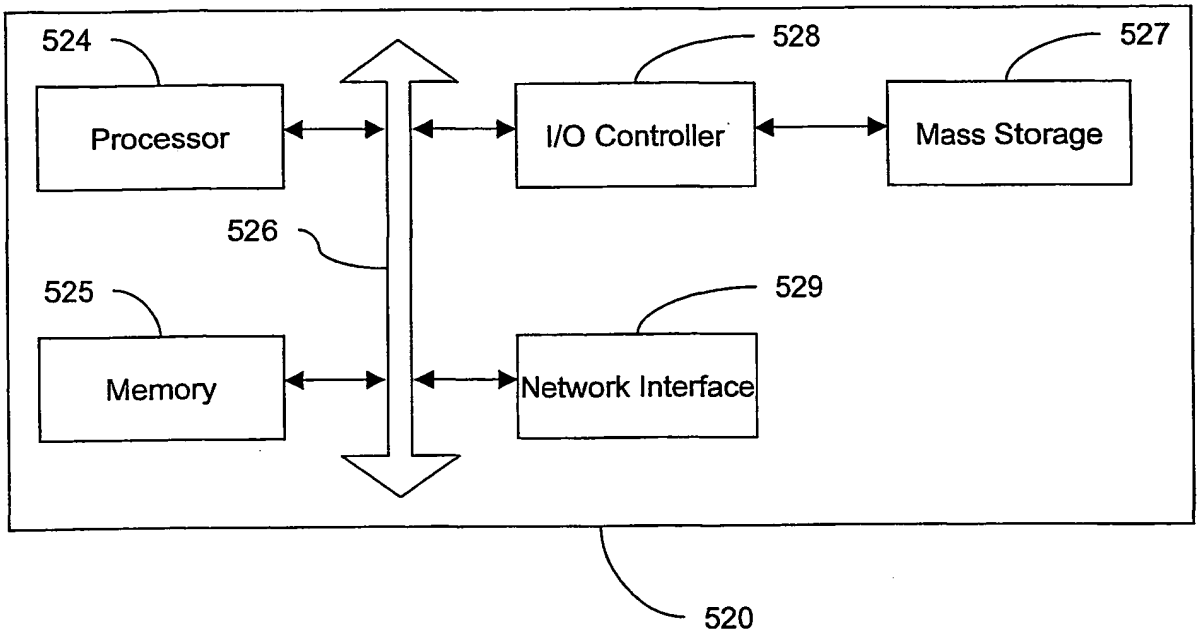

Desktop System
Desktop System
410
420
Network
400
412
Server A
Server B
422
435
Desktop System
425

Figure 5


524
528
527
Processor
I/O Controller
Mass Storage
526
525
529
Memory
Network Interface
520

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(25) Filing Language: English

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(30) Priority Data:
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(81) Designated States *(national)*: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, PH, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, UZ, VN, YU, ZA, ZW.

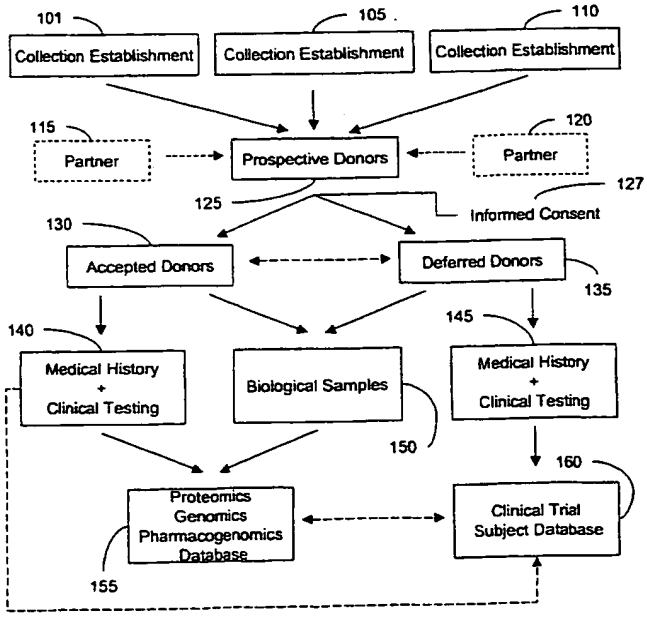
(84) Designated States *(regional)*: ARIPO patent (GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, TR), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).

Published:
— *with international search report*
— *before the expiration of the time limit for amending the claims and to be republished in the event of receipt of amendments*

(88) Date of publication of the international search report:
13 June 2002

[Continued on next page]

(54) Title: USE OF BLOOD AND PLASMA DONOR SAMPLES AND DATA IN THE DRUG DISCOVERY PROCESS



(57) Abstract: Systems consistent with the present invention provide a method for identifying and recruiting donors whose demographic characteristics, genomic and proteomic profile, and medical histories make them attractive candidates for clinical trials, drug target identification, and pharmacogenomic studies.

WO 02/17770 A3

For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US01/26598

A. CLASSIFICATION OF SUBJECT MATTER
IPC(7) :A61M 1/00
US CL : 604/30,31,66, 67
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 604/30,31,66, 67

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

STN on line

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	US 5,558,638 A (EVERS et al.) 24 September 1996, entire document.	1-45,49-61, 66-68
Y	US 5,970,499 A (SMITH et al.) 19 October 1999, claims 1-74.	1-45,49-61, 66-68
Y	US 5,809,493 A (AHAMED et al.) 15 September 1998, claims 1-20.	1-45,49-61, 66-68

☐ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

*	Special categories of cited documents:	"T"	later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"A"	document defining the general state of the art which is not considered to be of particular relevance	"X"	document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"E"	earlier document published on or after the international filing date	"Y"	document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"L"	document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	"&"	document member of the same patent family
"O"	document referring to an oral disclosure, use, exhibition or other means		
"P"	document published prior to the international filing date but later than the priority date claimed		

Date of the actual completion of the international search 14 JANUARY 2002	Date of mailing of the international search report 18 MAR 2002
Name and mailing address of the ISA/US Commissioner of Patents and Trademarks Box PCT Washington, D.C. 20231 Facsimile No. (703) 305-3230	Authorized officer Jayce Bridgers for MICHAEL BORIN Telephone No. (703) 308-0196

Form PCT/ISA/210 (second sheet) (July 1998)*

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US01/26593

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This international report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

Please See Extra Sheet.

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☒ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:
1-45,49-61,66-68

Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest.

☐ No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US01/26593

BOX II. OBSERVATIONS WHERE UNITY OF INVENTION WAS LACKING
This ISA found multiple inventions as follows:

This application contains the following inventions or groups of inventions which are not so linked as to form a single general inventive concept under PCT Rule 13.1. In order for all inventions to be searched, the appropriate additional examination fees must be paid.

Group I, claims 1-45, 49-61, 66-68 drawn to method of identifying a research subject.
Group II, claims 46-48, drawn to a set of biological samples.
Group III, claims 62-65, drawn to method of identifying correlation of a disease with genomic or proteomic information.

The inventions listed as Groups I-IV do not relate to a single general inventive concept under PCT Rule 13.1 because, under PCT Rule 13.2, they lack the same or corresponding special technical features. Unity of invention exists only when there is a technical relationship among the claimed inventions involving one or more special technical features. The term "special technical features" is defined as meaning those technical features that define a contribution which each of the inventions considered as a whole, makes over the prior art.
The method of Group I does not require the particular set of samples of Group II, Group I (as in claim 24) does not require plurality of samples, as required in claim 46, and there is nothing in the claims that identifies the particular set of samples of claim 46 as a special technical feature. Further, the latter set of samples would not be considered as a "special technical feature" as such samples are routinely obtained from any patient. Similarly, Group III utilizes database created using samples of Group II (note that Group II is drawn to samples rather than to a particular database created on the basis of these samples; the latter set of samples would not be considered as a "special technical feature" as such samples are routinely obtained from any patient).

This application contains claims directed to more than one species of the generic invention. These species are deemed to lack unity of invention because they are not so linked as to form a single general inventive concept under PCT Rule 13.1. In order for more than one species to be examined, the appropriate additional examination fees must be paid. The species for each group are as follows:

A) medical data comprise medical /family history or clinical chemistry;
B) medical data comprise genomic data;
C) medical data comprise proteomic data